ENHANCEMENT OF VEGETATIVE GROWTH IN YOUNG CITRUS PLANTINGS

BY

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Thesis presented in partial fulfilment of the requirements for the degree Master of Science in Agriculture in the department of Horticultural Science, University of Stellenbosch, Stellenbosch

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March 2000

Declaration

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously, in its entirety or in part, been submitted at any university for a degree.

Signature Date

Summary

Enhancement of vegetative growth in young citrus plantings

The establishment of citrus involves high input costs, with break-even usually only attained after six years, making early returns imperative for economic survival. Early production is inseparably associated with good growth of the trees in the non-bearing years.

In cool and cold production regions, coupled with a high frequency of wind, the initial tree growth is unsatisfactory. Trials were conducted to evaluate the effect of foliar application of Progibb® (GA_3), Promalin® (GA_{4+7} + BA), Kelpak® (seaweed extract containing auxins and cytokinins) and soil applications of Temik® (aldicarb) as a means to promote vegetative growth. GA_3 and/or aldicarb significantly increased tree height, without increasing the stem diameter in comparison to the control trees on 'Eureka' lemon, but not on 'Lisbon' lemon. GA_{4+7} + BA or Kelpak® treatments did not have an effect on tree growth or fresh weight distribution. In conclusion, growth stimulation by GA_3 and/or aldicarb could lead to quicker filling of the allotted space, and consequently higher, earlier yields, but further trials are required to verify these results.

Insufficient carbohydrate and nitrogen reserves are often implicated in poor establishment performance of nursery trees. The effect of tree size at planting and different topping heights on early growth in the field were evaluated; also the physical and chemical profile of tall nursery whip trees were quantified. Significant correlations were found between the initial stem diameter and final stem diameter and initial tree height and final tree height at the end of the first growing season. Stem

diameter increase was significantly reduced in topped trees relative to untopped trees. Roots comprised approximately 22%, and the aboveground portion about 78% of total dry weight. Roots nearly always contained higher concentrations of carbohydrates and nitrogen reserves. However, the aboveground sections, comprising a higher proportion of total dry weight, contained more than two thirds of total carbohydrates and nitrogen reserves. Topping of nursery whip trees at 60 cm aboveground thus would result in a loss of 33 to 37% dry matter, 29 to 33% carbohydrates and 37 to 46% nitrogen. Therefore, planting large, untopped nursery trees enhance initial tree growth in the orchard.

The use of well-branched nursery trees for orchard planting can shorten the time to commercial fruit production. Citrus nursery trees do not develop lateral shoots adequately in the nursery or in the field and therefore necessitate various branch induction techniques. Two trials on newly-planted (one or five months after planting) trees were conducted to evaluate various branch induction techniques, viz., girdling, Promalin® (GA_{4+7} + BA) (at 1000 or 2000 mg/*l*) and/or leaf removal and notching either to individual buds or the whole trees. The first trial, on 1-month-old trees did not yield significant results. In the second trial, notching and girdling significantly increased the number and total length of lateral branches, but notching was the most effective technique. As the first trial, on the very young trees, yielded no significant results, it seems that trees have to be well established before they will respond.

In young, non-bearing trees a lot of energy is invested in the flowering process, which results in an inhibition of vegetative growth. Gibberellic acid (GA_3) and heavy mineral oil (Bac-oil) treatments, either individually or in combination, applied during

early winter were evaluated as a means to reduce flowering levels in young non-bearing citrus trees. GA_3 and mineral oil either separately or in combination were sprayed from April to July to the whole tree. GA_3 and mineral oil either individually or in combination applied late in May to late June markedly reduced flowering. GA_3 application in mid April had a minimal effect on flowering levels. Peak responses to GA_3 coincided with a significant reduction in bud sprouting. Although optimum application time proved to be around May to July, this time is likely to vary from year to year depending on the influence of the prevailing temperature and other climatic conditions. The winter application of GA_3, specifically, and possibly in combination with mineral oil to inhibit flowering and early vegetative growth may be utilised commercially. However, response may vary dramatically from season to season.

Effect of time of application and concentration of 2,4-dichlorophenoxypropionic acid (2,4-DP) and 1-naphthaleneacetic acid (NAA) as potential fruit thinning agents to reduce fruit set soon after flowering and enhance vegetative growth were evaluated over a two year period. 2,4-DP at 150 and 300 mg/*l* and NAA at 200 and 400 mg/*l* were sprayed on 2- and 4-year-old 'Mihowase' Satsuma, as well as on 2-year-old 'Marisol', 'Nules' and 'Oroval' Clementine trees at two times (late October and early November). The higher concentration of 2,4-DP and NAA generally did not result in increased fruit abscission in the first year. However, in the case of 2,4-DP the higher concentration resulted in stronger thinning in the second year. 2,4-DP and NAA can be used as potential fruit thinning agents to reduce fruit set on young non-bearing trees.

Opsoming

Verbetering van vegetatiewe groeie by jong sitrus-aanplantings

Die vestiging van sitrus behels hoë insetkoste en die gelykbreekpunt word gewoonlik eers na ses jaar bereik. Vroë opbrengste is dus noodsaaklik vir ekonomiese oorlewing.

In koel en koue produksie-areas, met baie wind, is die aanvanklike boomgroei onvoldoende. Studies is uitgevoer om die effek van blaarbespuitings van Progibb® (GA_3), Promalin® (GA_{4+7} + BA), Kelpak (seewier-ekstrak wat ouksien en sitokiniene bevat) en grondtoedienings van Temik® (aldicarb), as metodes om vegetatiewe groei te verbeter te evalueer. GA_3 en/of aldicarb het boomhoogte betekenisvol laat toeneem, sonder om stamdeursnit te bevoordeel op 'Eureka' suurlemoen, maar nie op 'Lisbon' suurlemoen nie. GA_{4+7} + BA of Kelpak® behandelings het geen effek op boomgroei of varsmassa-verspreiding gehad nie. Groeistimulasie deur GA_3 en/of Aldicarb kan lei tot vinniger vul van spasie, en hoër en vroeër oeste, maar verdere studies is nodig om die resultate te bevestig.

Onvoldoende koolhidraat- en stikstofreserwes word dikwels gekoppel aan swak vestigingsprestasie van kwekerybome. Die effek van boomgrootte by planttyd en verskillende tophoogtes op vroeë groei in die veld is geëvalueer; die fisiese en chemiese profiel van lang ongetopte kwekerybome is ook gekwantifiseer. Betekenisvolle korrelasies is gevind tussen die aanvanklike stamdeursnit en en finale stamdeursnit en tussen aanvanklike boomhoogte en finale boomhoogte aan die einde van die eerste groeiseisoen. Stamdeursnit-toename is betekenisvol verminder in

getopte bome relatief tot ongetopte bome. Wortels het uit ongeveer 22% en die bogrondse porsie ongeveer 78% van die totale droë massa bestaan. Wortels het bykans altyd hoër konsentrasies koolhidrate- en stikstofreserwes bevat. Die bogrondse gedeeltes, wat ook 'n hoër proporsie van die totale droë massa bevat, het meer as twee derdes van die totale koolhidraat en stikstofreserwes. Die top van kwekerybome op 60 cm bo die grond sal lei tot 'n verlies van 33 tot 37% droë massa, 29 tot 33% koolhidrate en 37 tot 46% stikstof. Dus, die plant van groot, ongetopte kwekerybome sal die aanvanklike boomgroei in die boord verbeter.

Die gebruik van goedvertakte kwekerybome by planttyd kan die tyd tot kommersieële vrugteproduksie verkort. Sitrus-kwekerybome gee nie voldoende laterale vertakking in die kwekery of in die boord nie. Twee studies op pas-aangeplante bome (een en vyf maande na plant) is uitgevoer om verskillende tegnieke om laterale lootgroei te stimuleer te evalueer, nl. Promalin® (GA_{4+7} + BA) (teen 1000 of 2000 mg/*l*) en/of blaarverwydering, en die maak van kerfies op individuele knoppe of op bome as geheel. In die eerste studie, op die een-maand-oue bome, is geen betekenisvolle resultate verkry nie. In die tweede studie het kerfies en ringelering die hoeveelheid en lengte van laterale takke betekenisvol vermeerder, maar kerfies was meer effektief. Die bome moet egter blykbaar goed gevestig wees voor dit reageer.

In jong, nie-draende bome word baie energie gebruik in die blomproses en dit lei tot die inhibisie van vegetatiewe groei. Gibberelliensuur (GA_3) en 'n swaar mineral olie (Bac-oil) wat individueel of in kombinasie toegedien is tydens die vroeë winter is geëvalueer as 'n tegniek om blomvlakke in jong nie-draende sitrusbome te verminder.

GA_3 en minerale olie individueel of in kombinasie, is gespuit vanaf April tot Julie op die bome as geheel. GA_3 en minerale olie toegedien individueel of in kombinasie laat in Mei tot Junie het blomvlakke verminder. GA_3 toegedien in middel April het 'n minimale effek gehad. Die beste reaksie op GA_3 het saamgeval met 'n betekenisvolle vermindering in die bot van knoppe. Alhoewel die optimum tyd van toediening rondom Mei tot Julie is, mag hierdie tyd wissel van jaar tot jaar afhangende van die effek van heersende temperature en ander klimaatstoestande. Die wintertoediening van GA_3, spesifiek en moontlik in kombinase met minerale olie om blomvlakke en vroeë vegetatiewe groei te verminder kan kommersieel gebruik word. Die reaksie op hierdie behandelings mag egter drasties wissel van seisoen tot seisoen.

Die effek van die tyd van toediening en konsentrasie van 2,4-dichlorofenoksipropioonsuur (2,4-DP) en 1-naftaleenasynsuur (NAA) as potensiële vruguitdunagente om vrugset kort na blom totaal te verminder en om vegetatiewe groei te verbeter, is geëvalueer oor 'n twee-jaarperiode. 2,4-DP teen 150 en 300 mg/*l* en NAA teen 200 en 400 mg/*l* is gespuit op 2- en 4-jaaroue 'Mihowase' Satsuma, en ook op 2-jaaroue 'Marisol', 'Nules' en 'Oroval' Clementines op twee tye (laat Oktober en vroeg November). Die hoër konsentrasies van 2,4-DP en NAA het oor die algemeen nie gelei tot 'n verhoging in vrugafsnoering in die eeste jaar nie. Met 2,4-DP het die hoër konsentrasie gelei tot strawwer uitdunning in die tweede jaar. 2,4-DP en NAA kan as potensiële vruguitdunagente op jong, nie-draende bome gebruik word.

Dedicated to my mother, Vhengani and to the memory of my father, Vhangani

Acknowledgements

I gratefully acknowledge and thank the following individuals and institutions.

National Research Foundation and Citrus Research Committee for the generous financial support.

Special thanks to my supervisors, Prof. E. Rabe for guidance, constructive criticism, encouragement throughout my study and Prof. K.I. Theron for her constructive comments and invaluable assistance with the statistical analysis of the research data.

The lecturers and staff of the Department of Horticultural Science, University of Stellenbosch for their assistance, advice and encouragement throughout my study.

Messrs. G. Malherbe, A. Smit, R. Semple, F. Kirsten, K. Kirsten, M. T. le Roux, R. Allen, K. Watt and P. Turner for making available trial sites and their assistance in my trials.

Finally, thanks to my parents and family whose unwavering support is reflected in the success of this study.

CONTENTS

1. LITERATURE REVIEW

PHYSIOLOGICAL AND ENVIRONMENTAL REGULATION OF GROWTH AND DEVELOPMENT.

1.1 INTRODUCTION

Recently, citrus orchards are predominantly established with whip trees, especially in the Western Cape. In establishing an orchard, growers strive to (i) fill allotted space as quickly as possible, (ii) to develop a suitable framework to support the eventual fruit crop and (iii) to bring the tree into fruiting at the earliest possible date. The latter is usually more of a problem in the cool and cold production areas. The cost of establishment, especially at higher densities, necessitates quicker filling of the allotted space, and consequently earlier yields.

The quality of nursery trees to be planted in the orchard is a major factor determining not only the rate at which the orchard becomes established, but also the level of productivity in the early years (Van Oosten, 1978). The use of well-branched nursery trees for orchard planting can shorten the time to commercial fruit production (Quinlan, 1978). Thus, by increasing the number of actively-growing shoots, allotted space is filled early, more leaf surface is available for photosynthetic activity, generally enhancing tree canopy development.

Citrus trees in contrast with deciduous trees do not store large amounts of carbohydrates in their root system or trunks (Boswell and Cole, 1978). Rabe (1996)

found that high topping was advantageous to initial stem thickening. When topping a 1.5 m tall whip nursery trees at 60 cm aboveground a major percentage of reserves and dry matter are removed. It would therefore seem that retaining the nursery-produced reserves would be advantageous to translate into quicker tree growth and consequently larger, early yields.

While early production is one of the major goals, there is a need for regulating cropping during the establishment years. Cropping of young trees too early may negatively affect tree growth and therefore severely reduce growth in subsequent years. Inhibition of flowering and thinning of fruitlets in the early years would stimulate vegetative growth (Jones *et al.,* 1974; Guardiola *et al.*, 1982).

A better understanding of the interaction of environmental factors, hormones, and carbohydrate and nitrogen composition of citrus trees is important in order to devise reliable ways to enhance vegetative growth during establishment years. Therefore, the objective of this review is to address various factors, which may limit vegetative growth in young non-bearing citrus plantings.

1.2 Growth and development

1.2.1 Shoot and root growth

Shoot growth occurs in most types of citrus in several well-defined flushes (Spiegel-Roy and Goldschmidt, 1996). Under cool climatic conditions only two flushes appear annually, while three to five flushes occur in warmer, subtropical regions. For canopy

size increase the tree is dependent upon the summer flushes (Spiegel-Roy and Goldschmidt, 1996).

Citrus leaves start their expansion before the termination of shoot elongation. The leaf, which is initially light green reaches 80% of its full size within 1-2 months, and then becomes dark green and leathery (Spiegel-Roy and Goldschmidt, 1996). According to Kriedemann (1969), citrus leaves do not start exporting photosynthates until they are fully matured.

Leaf abscissions of orange trees take place throughout the year. The most intensive drop period occurs during the spring blossoming period, including old as well as young current-year leaves (Spiegel-Roy and Goldschmidt, 1996). A second, prolonged period of leaf abscission occurs during the fall (Erickson and Brannaman, 1960).

Root growth is a complex physiological process whereby the direct effects of environmental factors and endogenous mechanisms of the plant are combined, particularly in relation to shoot growth.

The root system for established field trees is reported to exhibit alternating growth flushes with the shoots (Bevington and Castle, 1985). Bevington and Castle (1985), observed that the rate of total shoot extension growth occurred simultaneously with the lowest rate of root extension of citrus trees. According to the theory, auxin, produced by the young shoot meristems is transported basipetally, stimulate growth of the roots and consequent cytokinin production. The acropetal cytokinin transport in

turn promotes shoot growth. This is in contrast to the finding Monselise (1947) who found that auxin inhibited root growth.

The growth periods may overlap, particularly in flushes which occur after the spring cycle since the growth of one component does not necessarily eliminate simultaneous growth by the other. A cyclic growth pattern is not unreasonable since each major organ is basically heterotrophic and competes for the available food supply (Castle, 1978). The normal distribution of plant assimilates become temporarily unbalanced to support the growing, more competitive organ. In trees with heavy crop loads root growth is completetly checked, presumably due to depletion of carbohydrates (Goldschmidt and Golomb, 1982).

Most of the differences in mineral nutrition and salt tolerance between rootstocks probably reflect distinct structural and physiological properties of the roots. Relatively high hydraulic conductivity of roots can increase the flow of water and mineral elements through the plant system to the leaves. Comparisons among citrus rootstocks have shown that rootstocks with high root conductivities generally results in more vigorous canopy growth. Thus, vigorous rootstocks, such as rough lemon and 'Carrizo' citrange tend to have higher root hydraulic conductivities than less vigorous stocks, such as 'Cleopatra' mandarin and sour orange (Syvertsen, 1981). Although there are differences in conductivities, there are no big difference in vigour among 'Carrizo', sour orange and 'Cleopatra' (Rabe, pers. comm.[1]).

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1.2.2 Interaction of reproductive and vegetative growth

Cameron (1932) and Maggs (1963) found that there is an inverse relationship between vegetative growth and fruiting. Young trees can be precocious and flower intensely, especially in cool and cold production regions. Under correct climatic conditions, flowers on young trees usually set heavily. There is a limited supply of resources within the tree for growth. The presence of fruit leads to competition for carbohydrate reserves between the fruit and the structural wood, roots and shoot growth. It is thus counter-productive for young trees to bear fruit before an adequate framework and a sufficient number of potential fruiting units have developed.

Guardiola *et al.* (1982) found that application of gibberellic acid (GA_3) during early November (N.H.) resulted in a significant inhibition of flowering and increase in the number of vegetative shoots in sweet orange. Deblossoming experiments by Maggs (1963) on two year-old trees showed greater dry-weight accumulation, increased leaf size, leaf number, and stem diameter in deblossomed than in fruiting trees. Jones *et al.* (1974) found increased vegetative growth when all fruits were removed three months after bloom in 'Valencia' orange trees. 1-naphthalene acetic acid (NAA) is widely used for thinning apples and citrus. However, application of NAA to 'Empire' apple suppressed CO_2 assimilation and possibly photosynthetic efficiency for more than two weeks with complete recovery after two to three weeks after the treatment (Stopar *et al.*, 1997).

1.2.3 Physiological factors affecting apical dominace

Sylleptical branching of a number of citrus cultivars is not adequate in the nursery or in the field. When tall nursery whip trees are planted in the field, proximal buds do not readily sprout; instead they branch apically with the first flush. During subsequent flushes, lateral buds start flushing (Rabe, pers. comm.[2]).

The effects on bud position and the existence of a growth potential along the axis of shoot is related to apical dominance. Snow (1925) was the first to propose a hormonal signal to explain apical dominance. It has been suggested that auxin moves down the shoots and actively inhibits development of lateral buds (Thimann and Skoog, 1933), yet many problems about the proposed auxin signal exist. The main obstacle is the inability of the proposed IAA signal to move acropetally into the buds in order to inhibit them (Snow, 1937). Secondary factors or messenger such as ABA or ethylene have therefore been suggest to transmit the effect of the primary signal into the organ to be inhibited (Tucker, 1978; Hillman *et al.*, 1985). New results show that the polar IAA export of the earlier developed sink inhibits the IAA export of later developed sinks organs (Bangerth, 1989). This 'autoinhibition' occurs at 'junctions' where auxin streams from various sinks meet. It is suggested that this depressed IAA-export of the subordinate sink act as the signal that leads to inhibited development.

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Alternatively, it may represent a substrate-induced mechanism, which could explain the effect of ethylene and cytokinins. The accumulating IAA above the junction possibly stimulates ethylene production at a node (Burg and Burg, 1968) which could cause lateral growth inhibition. In contrast, cytokinins, possibly originating from roots, can inhibit IAA metabolism and promote lateral bud growth (Phillips, 1975; Hillman *et al.*, 1985). In tomato shoots, Tucker (1975) found high levels of IAA and ABA associated with the suppression of branching following far-red treatments. In 'rogue' tomato which has weaker apical dominance and more branching than normal plants, low levels of IAA and ABA were found (Tucker, 1977). However, Knox and Wareing (1984), did not find a decrease in ABA content of buds in decapitated plants until after the lateral buds had begun to grow. This casts doubt on a causal role of ABA in bud inhibition.

Others suggest that auxin, somehow directs nutrients, growth regulators, photosynthates, and other resources to actively-growing shoot tips, at the expense of other buds and shoots (Myers, 1990).

1.3 Reserves and their utilisation

1.3.1 Carbohydrate

All of the perennial organs of woody plants may serve a storage function (Loescher *et al.*, 1990), and for citrus this would include leaves. The highest concentration of carbohydrate reserves is usually found in root tissues (Loescher *et al.*, 1990), and in citrus that is no exception (Goldschmidt and Golomb, 1982). Starch is the important storage carbohydrate in citrus (Cameron, 1932). A considerable portion of dry matter

is deposited in the cell wall as cellulose, hemicellulose and lignin and is therefore not available for utilisation by the plant (Goldschmidt and Golomb, 1982).

In citrus, the soluble carbohydrate pool usually contains fructose, glucose, and sucrose as a major component, sucrose being a major transport sugar (Sinclair, 1984). Soluble carbohydrates other than sucrose that occur in small amounts in both roots and shoots include inositol, xylose, rhamnose, maltose, trehalose, arabinose, ribose, mannose, and galactose, as well as the sucrose derivatives raffinose, and stachyose (Loescher *et al.*, 1990).

The types of carbohydrates that accumulate in roots often differ from those in the aerial tissues. Starch is the predominant reserve in roots. Large quantities of sucrose are commonly found in the aboveground tissues. Fructose and glucose usually are present in the roots in higher concentration than sucrose, but usually in lower concentration in aboveground tissues (Loescher *et al.*, 1990).

Carbohydrate levels are always fluctuating in citrus and are associated with the new growth flush. Starch levels increase in the old leaves toward the end of winter and then drop concomitantly with the emergence of the spring flush (Jones and Steinacker, 1951; Sharples and Burkhart, 1954). Sharples and Burkhart (1954) found that seasonal trends of 1-year-old twigs were rather similar to those of leaves, although twigs retained somewhat higher levels of starch throughout autumn and winter. Starch accumulation takes place in roots throughout autumn and winter and seems to be highly dependent upon the demand made by the fruit (Shimizu *et al.*, 1978). As soon as the temperature increases in spring, but before new growth starts, there is a

conversion of starch into more readily usable soluble carbohydrates such as fructose, glucose, and sucrose (Sharples and Burkhart, 1954).

Cameron (1932) found that starch accumulated in the leaves and twigs of young orange trees during the winter, but no rapid or exhaustive depletions occurred at the time of spring growth. Jones and Steinacker (1951) on the other hand, reported that, each flush of 'Valencia' orange was preceded by accumulation of starch in the leaves, but there was a rapid depletion during the spring growth. Sharples and Burkhart (1954) found that initiation of spring growth and blossoming to be exhaustive of reserves carbohydrate in regions of the tree nearest to the meristem.

Soluble sugars increase towards mid winter in cool areas, as an osmotic, protective measure against cold injury of delicate tissues (Jones and Steinacker, 1951; Sharples and Burkhart, 1954; Young, 1970). However, Cameron (1932) found no apparent conversion of starch to sugars during winter months and concluded that there is no winter reaction in citrus similar to deciduous trees. Cameron (1932) suggested that the increase in sugars was partly due to continuous photosynthesis that occurred during winter and the failure to utilise or to convert it to starch.

There is considerable evidence that certain types of shoot systems depend largely on carbohydrate reserves whereas others depend on current photosynthesis as well. For bud-burst and early spring growth, deciduous trees are totally dependent upon their reserves (Loescher *et al.*, 1990). In evergreens, the presence of the previous seasons' foliage, which is still photosynthetically active, may at least partly alleviate the dependence upon carbohydrate reserves (Goldschmidt and Koch, 1996). Kriedemann

(1969) found that newly-emerged growth drew ^{14}C-labelled assimilates from both current photosynthate and stored substrate. $^{14}CO_2$ labelling experiments indicated that reserve carbohydrates were utilised mainly to support reproductive development, while old leaves supplied the needs for vegetative growth (Akao *et al.*,1981).

Citrus leaves do not begin exporting photosynthates until they are fully expanded (Kriedemann, 1969). One to two months or more may be required for the completion of this expansion (Schaffer *et al.*, 1987). In citrus, older leaves, being lower on the plant, contribute assimilates to the root system as well as to new shoots (Kriedemann, 1969).

1.3.2 Nitrogen

Nitrogen has long been considered a dominating nutritional factor in the growth and development of citrus trees. Most plants are able to utilise both ammonium and nitrate as nitrogen sources. Some investigators have advocated the superiority of nitrate over ammonium, while others have concluded that nitrogen sources make little difference. Ammonium and nitrate are both taken up by the roots, and assimilated in the roots and leaves. Nitrogen accumulates in fall and winter in the leaves, aerial woody parts and roots and reutilised for spring growth.

1.3.2.1 Nitrogen uptake and nutrition

A. Ammonium and nitrate nutrition

Citrus trees absorb both ammonium and nitrate (Kato, 1986). Absorption of these ions may be affected by pH, temperature, ion composition of the medium, light, and carbohydrate supply (Kato, 1986).

'Valencia' orange cuttings absorbed more ammonium than nitrate when trees where fed with ^{15}N-labelled ammonium and nitrate (Wallace, 1954). Although citrus trees absorbed more ammonium than nitrate in water culture, greater uptake of ammonium does not always result in better growth. In water culture experiments, nitrate nutrition showed better growth than ammonium (Yokomizo and Ishihara, 1973), although the former had higher nitrogen content in leaves than the latter. However, Stewart *et al.* (1961) found insignificant differences in the nutritional effects of both ions in a sandy soil for pineapple oranges.

B. Partitioning of absorbed nitrogen

Kato and Kubota (1982) examined the uptake and partitioning of ^{15}[N] nitrate of 'Satsuma' mandarin during the coldest month. They found that more than 90% of absorbed ^{15}N remained in the fine roots. ^{15}N translocation upwards began in late winter to early spring. Akao *et al.* (1978) observed that 70-75 % of the ^{15}[N] nitrate applied to 9-or 15-year- old 'Satsuma' trees in September (fall) was translocated to the top parts of the trees and preferentially to new-developing organs in spring. Wallace *et al.* (1954) found in 3-year-old 'Washington' navel orange trees fed with ^{15}[N] nitrate applied in June, 92% of the absorbed ^{15}[N] moved to the top parts. The

major factors affecting the partitioning of absorbed nitrogen in spring and fall may be the development of new organs and the prevailing temperature.

1.3.2.2 Nitrogen assimilation

A. Nitrate reduction

Kato and Kubota (1982) found that leaf fragments contained twelve times more nitrate reductase activity that fine root segments. In citrus trees, the nitrate reductase activity in leaves was higher than in roots (Kato and Kubota, 1982). Nitrate reductase is a substrate inducible enzyme in many plants, and there is evidence that it is the case in citrus trees (Shaked *et al.*, 1974). Kato (1980) found that, when feeding ^{15}N-nitrate, glutamic acid and asparagine were actively synthesised, but glutamine synthesis was comparatively low compared with that of ammonium feeding. Nitrate is reduced to ammonium and then incorporated into amino acids.

Molybdenum is a component of the nitrate reductase molecule (Salisbury and Ross, 1992). Enzyme activity is low in molybdenum-deficient plants, but generally increases rapidly when molybdenum is applied exogenously (Kato, 1986).

The activity of nitrate reductase, like that of most enzymes, is affected by temperature. Kato and Kubota (1982) found that maximum activity was found to be at about 33°C. The rate of decrease of activity was less marked at lower than that at higher temperature. Thus even at 0.5°C, nitrate reductase in fine roots and leaves were, 10 and 13% of the maximum activity. This reduction in activity was related to

decrease in nitrate uptake, the size of the nitrate pool, and its assimilation into amino compounds at low temperature (Kato and Kubota, 1982).

B. Ammonium assimilation

The primary route for the initial assimilation of ammonium is believed to be through the activity of two enzymes, glutamine synthetase and glutamine (amide):2-oxoglutarate aminotransferase (Kato, 1986).

In citrus trees, most nitrogen assimilation in ammonium-fed plants occur in the roots, especially in fine roots. Kato (1980) found that, when citrus trees were fed with ^{15}N-ammonium, glutamine and glutamic acid as well as asparagine were synthesised and asparagine was the most predominant amino acid. Asparagine probably acts as an ammonium trap to prevent ammonium toxicity and is the main form of transport nitrogen upward under conditions of ammonium nutrition (Kato, 1980). Amino acid biosynthesis depends on the supply of carbon skeletons derived from carbohydrate catabolism as found in apples (Titus and Kang, 1982). Availability of carbon sources may depend on reserve carbohydrate, the amount of carbohydrate supplied by the leaves, and the rate of carbohydrate catabolism. When the input of ammonium exceeds the assimilation (detoxification) rate, ammonium toxicity occurs (Kato, 1986).

1.3.2.3 Storage and seasonal changes of nitrogen

Kubota *et al.* (1974) reported that free asparagine, arginine, and proline were major storage compounds in young 'Satsuma' mandarin trees. Proline, arginine, and

asparagine accumulate in fall and winter and are used for spring growth. Arginine and asparagine are considered to be particularly important as nitrogen compounds. These compounds are related to the nitrogen levels in the tree as they are rich in nitrogen (Kubota *et al.*, 1974) and may serve as nitrogen compounds.

Leaves, aerial woody parts, and roots seem to function as nitrogen storage tissues in citrus trees (Kato, 1986). The total protein content of old leaves decreased during the spring and summer growth. However, in young developing leaves total protein increased rapidly. The end of the spring and summer flushes were followed by quick recovery of the initial protein (Moreno and Garcia-Martinez 1984). The bark and wood accumulated total N, soluble N and protein after August (S.H.) and the increase in soluble N was due to an increase in supply of proline originating from the leaves (Kato *et al.*, 1985). Soluble forms of asparagine, proline, and arginine were found to be the major nitrogen compounds that increased in roots during fall and winter (Kubota *et al.*, 1974).

Kato *et al.* (1984) found that nitrogen in new parts of 21-year-old 'Satsuma'mandarin was derived from old leaves (22%), aerial woody parts (40%) and roots (30%). Kato *et al.* (1984) further found that wood of large branches, the trunk and major roots were the main nitrogen reservoirs for new shoot development. The major forms of nitrogen used for developing new organs were protein (50%) and soluble nitrogen (42%), primarily proline, arginine and asparagine.

1.4 Environmental factors affecting growth

1.4.1 Temperature

Khairi and Hall (1976) found that shoot initiation of 'Frost navel' orange was doubled in warm soils (25°C) compared to cool soils (15°C). They also found that combination of hot day air temperature (35°C) and warm soil (25°C) resulted in significantly more shoots. It was further found that 35°C day air temperature resulted in greater shoot elongation than 25°C, but warmer soil resulted in significantly more stem and leaves dry matter. Lenz (1969) also found higher temperature (30°C day/25°C night vs. 24°C/19°C night produced more vegetative growth and growth cycles of 'Washington' navel trees. Root temperature also affects growth and development of citrus trees. Bevington and Castle (1985) found for 'Valencia' orange on 'Carrizo' citrange that most intense root growth occurred when soil temperatures were kept above 27°C.

Studies with Douglas Fir suggest that spring growth may be initiated by gibberellins exported from the roots (Lavender *et al.*, 1973). Atkin *et al.* (1973) found that the export of total cytokinin and gibberellin was greatest at root temperature of 28°C while the inhibitor (abscisic acid) export was lowest at 33°C in maize. When root temperature was lowered to 8°C, shoot growth (together with an increase in inhibitor level) was restricted, with concomitant decline in cytokinin and gibberellin export. It would appear that soil temperature influence bud-break through effects on the hormonal balance in the shoot. Thus temperature may affect synthesis and /or translocation of hormones from the roots to the shoots.

1.4.2 Light intensity

Light intensity also affects vegetative growth and development of citrus trees (Davies and Albrigo, 1994). Light intensity has a direct effect on net CO_2 assimilation provided other factors such as temperature, nutrition and water are not limiting. Net CO_2 assimilation increases linearly as photosynthetic photon flux density (PPF) increases from 0 to 700 μmol m^{-2} s^{-1}, which is 30% of full sunlight (Syvertsen, 1984). The longer a citrus leaf is at, or above light saturation, the greater the net CO_2 assimilation.

Orchard crops, on the other hand, tend to attain their maximum leaf area by mid-summer, but usually intercept no more than 60-70% of available light at full canopy and may take years to attain this level (Jackson, 1980). The slow build-up of canopy structure over the years necessitates the need to enhance leaf area index at an early stage of tree development.

1.4.3 Photoperiod

Young (1961) found that 'Red Blush' grapefruit trees exposed to 16 hours of photoperiodic light produced more growth than those exposed to 8 hours. The increased growth resulted from more shoots per plant and not an increase in shoot length. It is likely that the increased growth from 16 hours of photoperiodic light is probably due to increased photosynthetic activity.

1.4.4 Wind

When young trees are unsupported and sway in the wind, the tree trunk develops more caliper (in an effort to support the tree) at the expense of linear shoot extension (Perry, 1989). Hot winds tend to cause excessive transpirational losses and death of exposed leaves through dehydration. Continuous wind also prevents the development of proper scaffold branches (Wahl *et al.*, 1991). Thus shoot growth development is hampered, resulting in a delay in coming into production and initial reduced production capacity.

1.5 Cultural aspects

1.5.1 Nutrition

A number of studies have been conducted on fertiliser practices of young citrus orchard trees. In general, young non-bearing trees are fertilised more frequently than mature trees, using smaller amounts of material for each application (Davies and Albrigo, 1994). This is due to the relatively limited root zone, particularly of a newly-planted trees. Bester *et al.* (1977) studied application frequency of broadcast urea and liquid fertilisers on newly-planted 'Valencia' orange trees and found that trunk diameter was the same for trees fertigated six times per year compared with broadcasted fertiliser applied four times per year. Willis and Davies (1991) also found no effect on tree growth of 'Hamlin' orange when comparing fertigation and granular-type of fertilisation. However, proper managed fertigation programmes may reduce water pollution, particularly with regard to nitrate, without compromising

growth of young trees. In addition, once the fertigation system is in place cost of application is considerably less for fertigation than granular-type programmes.

Nitrogen is the most important element regulating growth of young citrus trees since during this time trees are growing very rapidly (Davies and Albrigo, 1994). Other macro-elements, particularly phosphorus and potassium, are usually added to the fertiliser mix but have less impact on vegetative growth than nitrogen (Davies and Albrigo, 1994).

1.5.2 Irrigation

It is essential that young trees be correctly irrigated, particularly trees originating from container nurseries. Irrigation must be applied to the root area at least twice weekly until roots have spread into the surrounding soil (Lee *et al.*, 1987).

Chartzoulakis and Michelakis (1992) found that when young orange trees were subjected to water stress between -0.05 and -1.5 MPa, canopy diameter, plant height, and trunk diameter were significantly reduced. The transpiration rate, stomatal conductance and photosynthetic rate were reduced. Since stomatal opening affect CO_2 assimilation, water stress sufficient to close stomata would be expected to depress photosynthesis.

ABA (abscisic acid) is well known to accumulate to high concentrations in plant tissues under water limited conditions. It is also speculated that ABA is involved in the growth responses of water stressed plants, mostly related to growth inhibition.

Pierce and Raschke (1980) reported that turgor levels seemed to be controlling ABA production. It was found that water stressed roots also synthesise more ABA. This ABA is transported via the xylem to the leaves, where it may cause stomata to close.

1.5.3 Pests and Diseases

A healthy, functional leaf surface is essential for CO_2 assimilation and good growth. Any damage to the tips of new shoots could result in loss of growth (Lee *et al.,* 1987). The most common insect and disease problems of young trees are termites, orange dog caterpilars, psylla, thrips, budmite, aphids, scale insects, nematodes and *Phytophthora* (Lee *et al.*, 1987). Good pest and disease control is essential through all non-bearing years.

1.5.4 Tree support

In the citrus industry of South Africa, the concept of tree support has been linked with the trend of growing nursery whip trees that cannot stand upright by itself especially in windy regions like the Western Cape. Tree support has been hypothesised to increase shoot growth by decreasing secondary growth and therefore changing allocation of carbohydrates (Elfving and Mckibbon, 1992), due to reduced ethylene evolution (Perry, 1989).

Without tree support, the deciduous fruit grower will need to head the central leader severely for the first 3 – 4 years and resulting in a lower yield (Perry, 1989). By staking, heading in the terminal area is unnecessary except when stimulation of

branching is needed. The stake also provides rigid support for the terminal area, which stabilises the leader during bending and weighting of laterals to induce productivity in the developing tree.

1.6 Effect of pruning on tree growth

1.6.1 Pruning at transplanting

Traditionally, whip trees are topped at the desired height at transplanting. This is done for the following reason: (1) to allow the tree to develop without the detrimental effects of wind on the existing canopy (Foguet and Barry, 1995); (2) to allow the tree to develop framework branches in the field; (3) and to restore a more favourable root / shoot ratio at transplanting (Lee *et al.*, 1987).

Deciduous trees store a large amount of carbohydrate in their root system and can tolerate heavy pruning without a significant reduction in food reserves. Citrus trees on the other hand do not store large amounts of carbohydrate in their root system (Boswell and Cole, 1978). Instead, carbohydrates gradually accumulate in the leaves so that a maximum level is reached just before spring activity. Therefore, severe pruning of citrus trees can cause a considerable loss in carbohydrate. Rabe (1996) also found a clear advantage of higher topping on initial stem thickening. Further investigations showed that topping of 1.5 m tall whip trees at 60 cm result in the loss of 38 % dry matter, 73 % of photosynthetic capacity (new leaves) and 43 % carbohydrates reserves. Also, nitrogen reserves are lost. This may explain the faster initial growth of trees topped at higher positions (Rabe, 1996).

1.6.2 Pruning of young, non-bearing trees

The main objective in pruning of young trees is to develop a well-spaced scaffold branch structure (McCarty, 1967). Pruning young trees may increase the time for the trees to come into bearing. Tibshraeny (1995) found that severe manipulation during the early years reduces vegetative growth, canopy development and consequently, yield potential. If the orchard is planned as a long-term investment, pruning to form well-shaped trees may be worthwhile (McCarty, 1967). Shaping of soft citrus trees can be achieved by reducing the number of scaffold branches and ensuring that branch angles are wider (Wahl and Rabe, 1991). This pruning should not be heavy, but should be sufficient to establish the future scaffold branches.

It is emphasised that pruning should be held to a minimum. Although the grower is sacrificing immediate fruiting, he nevertheless has a tree with a better branch structure suited to fruit production (McCarty, 1967). An alternative will be to develop a well-formed tree in the nursery in order to avoid any further pruning of young trees in the field. The use of growth regulators may be an option to induce proper scaffold branches in the nursery.

1.7 Tree quality at planting and manipulation

1.7.1 Tree size

In horticulture, the form and size of nursery trees is of prime importance. In the deciduous industry it is generally accepted that feathered and/or large caliper nursery trees are of good quality (Sander, 1993). Marler and Davies (1987) found that

'Hamlin' orange nursery tree size is corrected positively growth during the first 20 months in the field.

There is considerable published data from the deciduous fruit industry to support the belief that larger caliper nursery trees continue to be large in the orchard and that small ones continue to be smaller. Rabe (1997) found that good elongation growth translates into the best stem diameter in young citrus nursery trees.

Container-grown trees are generally smaller in diameter than field-grown trees (Davies and Albrigo, 1994). Marler and Davies (1987) found that bare-rooted 'Hamlin' orange nursery trees were larger than container-grown trees 8 and 20 months after planting. They further found that removal of the medium from the container-grown trees upon transplanting improved the orchard growth during the first season. Rabe (1997) on the other hand did not find a significant initial growth difference after media removal to different levels, in 'Nules' Clementine trees, when combined with adequate irrigation practices.

The slower growth observed in container-grown trees may have resulted from inadequate wetting of the media surrounding the roots. Studies suggest that water movement from field to organic media may be slow (Marler and Davies, 1987). Some growers believe that bare-rooted trees grow faster because of their spreading, extensive root system but this is probably not the case.

1.7.2 Chemical manipulation to stimulate lateral branching

A. Promalin and benzyl-adenine

Veinbrants and Miller (1981) and Williams and Billingsley (1970) have shown that Promalin®, a commercial mixture of gibberellins (GA_{4+7}) and the benzyl-adenine (BA), caused lateral branching with a wider crotch angle on one-year-old wood of young cherries and apples trees. Koen *et al.* (1989) and Quellete *et at.* (1996) also found also that Promalin® increased branching of apple trees, but significantly reduced tree height.

Wright (1976) found that foliar application of BA to *llex crenata cvs*. Helliri and Rotundifolia increased the number of axillary shoots per plant.

Williams and Billingsley (1970) suggested that (GA_{4+7}) may cause the BA-induced branches to elongate, but the role of (GA_{4+7}) is not clear since BA alone has given similar results to Promalin®. Stimulation of cell division by cytokinin may be responsible for the wide crotch angles (Williams and Billingsley, 1970).

B. MB 25-105 (propyl 3-butylphenoxyacetate)

Strydom and Honeyborne (1980) found that, MB 25-105 increased sylleptic branch development of 'Topred' apple nursery trees, with higher concentration increasing the total number of laterals. MB 25-105 acts by reducing basipetal translocation of auxin from the shoot tip.

No data on chemical branch induction in citrus nursery trees could be found in the literature.

1.7.3 Physical manipulations to stimulate lateral branching

A. Heading

Nursery trees are topped at 60 cm or less in cases of scions that need scaffold branches at a lower level (Tibshraeny, 1995). Cutting back the top may be needed to bring the tree into balance with the reduced root system (Foguet and Barry, 1995).

Heading is a practical and economic method for the majority of newly-planted trees in Western Cape conditions where the trees are planted as unbranched whips. However, when heading the tree for field planting, only the buds close to the cut grow. These sprouting buds produce upright branches which bend over to form laterals (McCarty, 1967). This system is appropriate because it encourages a number of vigorous shoots for selection as good scaffold branches. However, the effectiveness of heading in stimulating scaffold branches depends on the vigour and natural growth habit of the tree.

B. Bending

Bending can be used to induce development of lateral shoots in deciduous trees (Strydom and Honeyborne, 1980). To obtain budbreak on all sides of the shoot, the shoot should be bent in one direction for a week and in the opposite direction for another week. This method can be useful in forcing laterals on whip trees.

Bending usually results in the laterals only developing on the dorsal side of shoots (Strydom and Honeyborne, 1980). Gravity probably influences the auxin orientation down the shoot and the buds on the upper parts are released from apical dominance and start growing.

C. Notching

Notching above buds has been used to stimulate growth of individual buds (Greene and Autio, 1994) in deciduous trees. Notches can be made with a hacksaw, blade by removing a 2 mm wide strip of bark about 5 mm above the bud (Greene and Autio, 1994). Greene and Miller (1988), found that shoots that develop from notched buds have a narrow crotch angle than shoots that develop from buds that were not notched. However, when BA was combined with notching, crotch angles were increased.

Auxin moving from young leaves in the shoot tip is thought to serve as the principal correlative signal in bud inhibition (Quinlan, 1978). Notching stimulates lateral branching by interrupting the downward movement of auxin (Tamas, 1987). As long as the phloem remains severed and auxin is prevented from reaching the lateral bud, growth and development of the bud will not be inhibited (Greene and Autio, 1994).

D. Defoliation

Removal of immature, apical leaves has been reported to increase the number of branches in some apple cultivars (Wertheim, 1978). Theron *et al.* (1987) and Popenoe and Barrit (1988) found that removing young leaves stimulated axillary bud

growth, while removing older leaves had less effect. Quellete *et al.* (1996) found that combining leaf removal and Promalin® resulted in more uniform distribution along the length of the central leader.

Conclusion

The available information suggests that development of a plant is controlled by physiological and environmental factors. Environmental factors (controllable and non- controllable) are important in allowing or preventing growth. However, they operate through their influence on hormonal factors and mobilisation of reserves for vegetative growth.

From the pruning studies, initial tree growth during the establishment years seems to be quantitatively correlated with carbohydrates and nitrogen availability (McCarty, 1967; Rabe, 1996). By saying this we do not deny the involvement of plant hormones and other regulatory systems in the control of growth and development.

There is still lack of sufficient understanding of the hormonal control of plant growth and development. Most of the basic physiological studies on apical dominance have been conducted on deciduous or herbaceous plants. Similar studies are needed on citrus. When that is clear, we probably will be able to devise ways to either enhance vegetative growth or to manipulate branching in young citrus trees via the use of the plant growth regulators and/ or by physical means.

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2. PAPER 1: ENHANCEMENT OF VEGETATIVE GROWTH ON NEWLY-PLANTED CITRUS TREES BY GROWTH-PROMOTING CHEMICALS.

Abstract

In cool and cold production regions, coupled with a high frequency of wind, initial tree growth is unsatisfactory, thus delaying the break-even point. Foliar application of Progibb® (GA_3), Promalin® (GA_{4+7} + BA), Kelpak® (seaweed extract containing auxins and cytokinins) and soil applications of Temik® (aldicarb), either individually or in combination with GA_3, were evaluated as a means to promote vegetative growth. Treatments were applied at two dates, either singly or as repeated applications. In a second trial, Kelpak® was applied as a soil drench either singly or as repeated applications. GA_3 and/or aldicarb significantly increased tree height, without increasing the stem diameter in comparison to the control trees on 'Eureka' lemon, but not on 'Lisbon' lemon. GA_{4+7} + BA or Kelpak® treatments did not have an effect on tree growth or fresh weight distribution within trees. In conclusion, growth stimulation by GA_3 and/or aldicarb could lead to quicker filling of the allotted space, and consequently higher, earlier yields, but further trials are required to verify these results.

Introduction

The establishment of citrus involves high input costs, with break-even usually only attained after six years. In cool and cold production regions, coupled with a high frequency of wind, the initial tree growth is unsatisfactory and the enhancement of vegetative growth may facilitate earlier production.

Endogenous growth regulators play a key role in the growth and development of fruit trees. Gibberellins promote vegetative growth of many species (Salisbury and Ross, 1991). Exogenous application of gibberellic acid (GA_3) increases stem elongation of citrus trees (Monselise and Halevy, 1962; Ben-Gad *et al.*, 1979; Mehouachi *et al.*, 1996; Rabe, 1997).

Plant growth stimulation has been reported with the use of aldicarb and other pesticides in the apparent absence of pests (Bromilow *et al.*, 1980; McEwen and Cockbain, 1979; Nayeem and Bapat, 1984; Bakker *et al.*, 1988). Aldicarb promotes shoot growth of apple even though it suppresses photosynthesis and transpiration (Ferree and Hall, 1978). On the other hand, Womack and Schuster (1986), found that aldicarb suppresses growth of cotton plants.

A commercial seaweed preparation can improve plant growth (Metting *et al.*, 1991). Many of these effects have been attributed to the presence of growth substances, particularly cytokinins (Featonby-Smith and Van Staden, 1984). However, auxins have also recently been identified in a commercial preparation (Crouch *et al.*, 1992).

The application of seaweed concentrate to plants, has been reported to significantly increase root initiation and growth (Featonby-Smith and Van Staden, 1984). Both endogenous and synthetic auxins stimulate rooting (Jackson and Harney, 1970), while cytokinins inhibit rooting (Van Staden and Harty, 1988). Therefore, the possibility exists that the observed rooting response following seaweed application is due to auxins. An improvement of root growth soon after planting should translate into enhanced vegetative growth. The objectives of these trials were thus to evaluate various growth-promoting chemicals on newly-planted trees to enhance initial tree growth.

Materials and Methods

Plant materials. Three trials, using 'Lisbon' lemon, 'Eureka' lemon and 'Mihowase' Satsuma on 'Swingle' citrange, rough lemon, and 'Carrizo' citrange rootstocks, respectively, spaced at 5 x 2 m (both lemons) and 4.0 x 0.75 m (Satsuma) were conducted. The trials were conducted in the Wellington ('Lisbon'), Simondium ('Eureka') and Stellenbosch regions ('Mihowase'), respectively, (Western Cape, 34°S 19°E, ca. 100 m above sea level, mediterranean climate). Individual trees were selected for uniformity of size and vigour and trials commenced one or two months after planting.

Treatments and statistical design

Trials 1 and 2

Treatments consisted of: untreated control, foliar application of Progibb® (GA_3), at 50 mg/*l* water, Promalin® (GA_{4+7} + BA) at 50 mg/*l* water, Kelpak® was diluted 1:250 water (Trial 2 only) and soil applications of aldicarb at 20 g, either individually or in

combination with GA_3. Treatments were applied at two dates, singly or repeated in November 1997 and January 1998 (Trial 1) or February and March 1998 (Trial 2). Agral 90 at 0.2 m*l*/*l* was used as a wetting agent with the foliar sprays. The spray solution was applied to the entire trees as a full cover spray until run-off, using a hand-held spray bottle. Aldicarb was applied to the soil surface ±0.5 m^2 around the tree stem. The treatments were replicated 10 times in a randomised complete block design using two-tree-plots.

Trial 3

Kelpak® was applied as a soil drench at five dates, either as single or repeated applications at 18 or 36 day intervals. Kelpak® concentration was diluted 1:250 and control plants received only water. Every plant received 1.9 *l* of solution, saturating the entire root volume at each occasion. The treatments were replicated 10 times in a randomised complete block design using two-tree-plots.

Data recorded. Tree height and stem diameter was measured 5 cm above the bud union (Trials 1 and 2) or 50 cm aboveground level (Trial 3). At the end of the first summer, half of the plants of Trial 3 were destructively harvested to determine the number and length of lateral branches, and total fresh weight of roots and shoots.

Data analysis. The General Linear Models (GLM) procedure of the Statistical Analysis System (SAS Inc. 1990) was used to analyse the data.

Results

Tree height increase. A single application of GA_3 or two applications of GA_3 in combination with aldicarb resulted in the best tree height increase (31 – 37%) on 'Eureka' lemon (Table 1). Aldicarb (single or repeated applications) also enhanced tree height by more than 10% above the control, but this was not a statistically significant increase (Table 1). Kelpak® or Promalin® did not have an effect in any trial (Tables 1, 2 and 3). No treatment successfully increased tree height in 'Lisbon' lemon (Table 2).

Stem diameter. Stem diameter increment was unaffected by treatments, although where tree height was increased significantly, stem diameter was slightly smaller as compared to control trees (Tables 1, 2 and 3).

Lateral branches. The application of Kelpak® to the roots did not influence lateral branching ($P>0.05$) (Table 3).

Total fresh weight of shoots and roots. All Kelpak® treatments resulted in no effect on the mass of shoots ($P>0.05$) (Table 3).

Discussion

A single application of GA_3 significantly increased tree height of 'Eureka' lemon. However, this was not reflected in a significant increase in stem diameter. The repeated application did not improve growth and results were obtained which were not significantly different from the control. The present results confirm previous

reports that GA_3 applied at 50 mg/*l* significantly stimulates tree height without increasing stem diameter (Rabe 1997). GA_{4+7} + BA or Kelpak® do not seem to increase tree growth. No significant results were obtained on 'Lisbon' lemon and 'Mihowase' Satsuma. The response of the different lemon cultivars differed, reflecting differences in their response to GA_3 and aldicarb. Alternatively, 'Lisbon' lemon trees could have experienced some form of water stress immediately after the treatments were applied.

Our results showed that aldicarb enhanced growth, confirming other reports (Bromilow *et al.*, 1980; McEwen and Cockbain, 1979; Nayeem and Bapat, 1984; Bakker *et al.*, 1988), and that aldicarb possesses plant growth regulator activity, however, the mechanism remains to be established. Of importance however, is the fact that aldicarb can be applied immediately after transplanting to enhance tree growth.

Kelpak® application to the roots did not influence tree growth or fresh weight increase. This could be due to insufficient amounts of Kelpak®, suggesting that higher concentrations may be required to stimulate growth. Alternatively, when growing conditions are optimum, trees may already contain sufficient amounts of auxins or cytokinins and therefore may not respond to Kelpak®.

In conclusion, growth stimulation by GA_3 and/or aldicarb could lead to quicker filling of the allotted space, and consequent higher, earlier yields, but further trials are required to verify these results.

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Table 1. The effect of GA_3, aldicarb, GA_{4+7} +BA and Kelpak on stem elongation and stem diameter of 2-month-old 'Eureka' lemon on rough lemon rootstock.

Treatment	Date of application	Tree height (cm)				Stem diameter (mm)			
		3/2/98	10/6/98	Increase	% control	3/2/98	10/6/98	Increase	% control
Control		104 a	161 ab	57 c	100	8.93 ab	15.00 a	6.08 a	100
50 mg/*l* GA_3	3 Feb.	99 a	176 a	78 a	137	8.92 ab	14.67 a	5.74 a	94
50 mg/*l* GA_3	3 Feb. & 20 Mar.	100 a	170 ab	70 abc	123	9.01 ab	14.73 a	5.72 a	94
20 g Aldicarb	3 Feb.	104 a	168 ab	64 c	112	8.56 ab	15.04 a	6.48 a	107
20 g Aldicarb	3 Feb. & 20 Mar.	104 a	174 a	70 abc	123	9.25 a	15.12 a	5.87 a	97
20 g Aldicarb & 50 mg/*l* GA_3	3 Feb. & 20 Mar.	100 a	175 a	75 ab	132	8.91 ab	14.36 a	5.45 a	90
50 mg/*l* GA_{4+7} + BA	3 Mar.	105 a	165 ab	60 c	105	8.91 ab	14.93 a	6.03 a	99
50 mg/*l* GA_{4+7} + BA	3 Feb. & 20 Mar.	99 a	156 b	57 c	100	8.73 ab	14.76 a	6.02 a	99
Kelpak (1:250)	3 Feb. & 20 Mar.	105 a	162 ab	58 c	102	8.32 b	14.36 a	6.44 a	106
LSD (0.05)		7	16	14		8.83	1.53	1.22	
Sign. level		0.3020	0.1973	0.0114		0.5738	0.3251	0.7824	

Table 2. The effect of GA_3, aldicarb, and GA_{4+7} + BA on stem elongation and stem diameter of 1-month-old 'Lisbon' lemon on rough lemon rootstock.

Treatment	Date of application	Tree height (cm)				Stem diameter (mm)			
		18/11/97	18/01/98	Increase	% control	18/11/97	18/01/98	Increase	% control
Control		137 a	178 a	41 a	100	8.64 a	15.48 a	6.84 a	100
50 mg/*l* GA_3	18 Nov.	142 a	181 a	40 a	98	8.92 a	14.63 ab	5.71 ab	83
50 mg/*l* GA_3	18 Nov. & 18 Jan.	134 a	175 a	41 a	100	8.67 a	14.76 ab	6.09 ab	89
20 g Aldicarb	18 Nov.	136 a	175 a	39 a	95	8.20 a	14.92 ab	6.72 ab	101
20 g Aldicarb	18 Nov. & 18 Jan.	140 a	181 a	41 a	100	9.05 a	14.95 ab	5.91 ab	86
20 g Aldicarb & 50 mg/*l* GA_3	18 Nov. & 18 Jan.	140 a	185 a	44 a	107	8.90 a	15.16 a	6.25 ab	91
50 mg/*l* GA_{4+7} + BA	18 Nov.	138 a	177 a	39 a	95	8.37 a	13.95 b	5.58 ab	82
50 mg/*l* GA_{4+7} + BA	18 Nov. & 18 Jan.	138 a	180 a	42 a	102	9.11 a	14.60 ab	5.49 b	80
LSD (0.05)		10	16	12		0.93	1.22	1.35	
Sign. level		0.8018	0.9259	0.9868		0.4674	0.3791	0.3596	

Table 3. The effect of Kelpak® as soil drench (1:250 concentration) on growth, lateral shoot formation and fresh weight distribution of 1-month-old 'Mihowase' Satsuma trees after 8-months of growth. Number in brackets represent application intervals

No. of applications and intervals	Tree height (cm)			Stem diameter (mm)			No of lateral shoots	Root fresh weight (g)	Total shoot fresh weight (g)	Total tree fresh weight (g)
	Intial	Final	Increase	Initial	Final	Increase				
0	135 a	161 a	26 ab	4.53 c	10.15 ab	5.62 a	9 a	113 a	247 a	360 a
1	136 a	149 a	13 b	4.76 bc	8.50 b	3.74 a	8 ab	105 a	199 a	304 a
2 (18)	134 a	156 a	22 ab	4.82 bc	9.53 ab	4.72 a	11 a	100 a	211 a	311 a
3 (18)	140 a	166 a	26 ab	4.67 bc	9.30 ab	4.63 a	11a	85 a	230 a	315 a
4 (18)	133 a	166 a	33 a	5.06 ab	10.49 a	5.43 a	6 b	100 a	265 a	365 a
3 (36)	136 a	161 a	25 a	5.29 a	9.64 ab	4.36 a	0 ab	104 a	256 a	360 a
LSD	7	18	19	0.44	1.20	1.89	4	33	79	79
Sign. level	0.4795	0.4132	0.4352	0.0172	0.4325	0.3723	0.1952	0.6544	0.4957	0.4279

3. PAPER 2: PHYSICAL AND CHEMICAL PROFILE OF CITRUS NURSERY TREES, EFFECT OF TREE SIZE AT PLANTING AND TOPPING HEIGHT ON INITIAL TREE GROWTH.

Abstract

There is considerable evidence that carbohydrate and nitrogen reserves in young trees play an important role in initial growth. The effect of tree size at planting and different topping heights on early growth in the field was evaluated; also the physical and chemical profile of tall nursery whip trees were quantified. Treatments consisted of various topping heights: i.e., 60, 80, 100, 120 cm aboveground and untopped trees (approximately 130 cm tall) using 'Eureka' lemon and 'Mihowase' Satsuma. Furthermore, 'Nules' Clementine and 'Eureka' lemon trees obtained from a commercial nursery were dissected into different components, i.e. roots (fine and major roots) and aboveground sections of: 0 – 60, 60 – 100, and 100 cm and more. Significant correlations were found between the initial stem diameter and tree height with stem diameter and tree height at the end of the first growing season. Stem diameter increase was significantly reduced in topped trees relative to untopped trees. Roots comprised approximately 22%, and the aboveground portion about 78% of total dry weight. Roots nearly always contained higher concentrations of carbohydrates and nitrogen reserves. However, the aboveground sections, comprising a higher proportion of total dry weight, contained more than two thirds of total carbohydrates and nitrogen reserves. Topping of nursery whip trees at 60 cm aboveground thus would result in a loss of 33 to 37% dry matter, 29 to 33% carbohydrates and 37 to 46% nitrogen. Therefore, planting of large, untopped nursery tree enhances initial tree growth in the field.

Introduction

Trees require reserves (carbohydrates and nitrogen) for initial growth establishment (McCarty, 1967). Usually, high reserve levels will translate into better initial growth.

Nurseries can produce tall whips (up to 1.5 m tall) and such trees, if established with a support structure, initially grow faster (Rabe, 1996). Marler and Davies (1987) found that larger nursery trees positively influence growth during the first 20 months of 'Hamlin' orange trees in the field. There is a considerable amount of data on deciduous fruit trees to support the belief that larger caliper trees continue to be a large tree in the orchard during the initial stage of development (Gardner and Horanic, 1959).

Nurseries participating in the Citrus Improvement Program of South Africa strive to produce a tree with physical dimensions of 8 mm stem diameter at 5 cm above the bud-union (Rabe, 1996). However, smaller caliper trees are still sometimes planted. Such trees may grow well under optimum conditions. However, in cool and cold production regions, when coupled with a high frequency of wind, the initial tree growth is slow and the smaller trees often do not perform satisfactorily during the establishment stage.

Usually, citrus trees are topped approximately 60 cm aboveground prior to planting, allowing formation of scaffold branches between 40 to 60 cm aboveground (Tibshraeny, 1995). This practice is done for the following reasons: to restore a more favourable root/shoot ratio at transplanting (Lee *et al.,* 1987) and also to allow the tree

to develop an initial framework of scaffold branches without having to support the tree. Deciduous trees store a large amount of carbohydrate in their root system and can tolerate heavy pruning without compromising vegetative growth too much (McCarty, 1967). Citrus trees on the other hand, being an evergreen, do not store large amount of carbohydrate in their root system (Boswell and Cole, 1978). Instead, carbohydrates gradually accumulate in the leaves so that a maximum level is reached just before spring activity. Levels of carbohydrate and nitrogen reserves are thus reduced following pruning. Pruning of nursery trees would reduced initial vegetative growth since a lot of the dry matter produced in the nursery is removed.

Once topped, the most apical branches assume a very upright habit, which suggests that it should have been left untopped from the outset. The aim of this study was to investigate: (1) the effect of tree size at planting on initial post-plant performance, (2) effect of different topping heights on early growth in the field; (3) also to quantify the physical and chemical profile of tall nursery whip trees to establish the levels of reserve loss upon topping prior to transplanting.

Materials and Methods

Tree size

Plant material. One-month-old 'Eureka' lemon on sour orange rootstock, spaced at 5 x 2 m, planted near Wellington (Western Cape, 34°S 19°E, ca. 100 m above sea level, mediterranean climate) were used.

Treatments, statistical design and data recorded. Fifty trees were selected at random. Stem diameter, 5 cm above the bud-union, and tree height were taken in November 1998 and again in April 1999.

Topping height

Plant material. 'Eureka' lemon on rough lemon rootstock (Trial 1, Simondium), spaced at 5 x 2 m and 'Mihowase' Satsuma on 'Carrizo' citrange rootstock (Trial 2, Stellenboch), spaced at 4.0 x 1.5 m were used. All trees were supported. Individual trees were selected for uniformity of size and vigour.

Treatments and statistical design. For the lemons (Trial 1), the treatments consisted of untopped trees or topping at 80 or 120 cm aboveground. The treatments were replicated 20 times in a randomised complete block design, using single tree plots. For Satsumas (Trial 2), the treatments consisted of untopped trees (approximately 130 cm tall), or topping at 60, 80, 100 or 120 cm aboveground. The treatments were replicated 10 times in a randomised complete block design using two-tree-plots.

Data recorded. In Trial 1, stem diameter was measured in February 1998 and March 1999, at 5 cm above the bud union. In Trial 2, stem diameter measurements were taken in December (1998) until August 1999 at 50 cm aboveground. After March 1999, trees in Trial 1 showed severe symptoms of root rot and further measurements were terminated.

Physical and chemical profile of nursery trees

Plant material. Two trials, one on 'Nules' Clementine (10 trees) and one 'Eureka' lemon trial (10 trees) were done. The 'Nules' Clementine trees were on 'Carrizo' citrange and 'Eureka' lemon on rough lemon rootstock. Trees were obtained from a commercial nursery. These trees, budded between 200 to 300 mm above the soil level, were between 1.4 to 1.9 m in height.

Sample preparation. The trees were sectioned into: (1) root (fine and major roots), (2) aboveground sections of: 0 – 60, 60 – 100 and 100 cm and more. The samples were stored at –20°C until freeze-dried, milled into a fine powder and stored in sealed containers at –20°C until analysed.

Carbohydrate (starch and total sugars) extraction and analysis. 0.5 gram dried, milled sample was extracted with 25 m*l* 1% acetic acid by shaking for ±14 hours in a 50 m*l* centrifuge tube and then centrifuged at 3000 r.p.m (600 g) for ten minutes. The supernatant was decanted into 100 m*l* volumetric flasks filled to volume with distilled water, and thereafter filtered through Whatman no.2 into sample bottles. The supernatant contained the sample soluble fraction. Total soluble sugars were determined on a Sanplus Segmented Flow Analysis System from Skalar using Method No. 551-965w/r issue 070798/MH.

The remaining pellet in the tube after decanting represents the insoluble residue containing the starch fraction, and was transferred to 100 m*l* volumetric flasks in a acetic buffer, pH 4.8 (0.2 N sodium acetate) and placed in boiling water at 100°C for two hours. Samples were cooled to below 60°C before addition of 50 µ*l*

amyloglucosidase enzyme (200 mg enzyme in 10 m*l* acetate buffer pH 4.8) for digestion of starch to glucose molecules by incubation at 50°C for 18 hours. Thereafter, flasks were made to volume with distilled water and filtered through Whatman no. 2 into sample bottles and stored at –20°C until further analysis. Starch was determined as glucose equivalents on a Sanplus Segmented Flow Analysis System from Skalar using Method No. 356–001w/r issue 012998/MH/97203066.

Total nitrogen analysis. 0.2 g dried, milled sample was weighed onto a standard white tissue paper and placed into a 100 m*l* digestion tube. The blank consisted of paper only. To each tube, 4 m*l* concentrated sulphuric acid, 2.5 g catalyst mixture (15 g copper sulphate, 250 g potassium sulphate and 27 g stearic acid mixed in powder form) and 2 m*l* peroxide (30-50% commercial) was added. The tubes were transferred to a mini-Kjeldahl heating block (preheated to 370°C) and left to digest for 30-60 min. until clear. The sides of the digestion tubes were rinsed down periodically with peroxide. The tubes were left to cool after removal, made to volume with distilled water and filtered through Whatman no. 2 paper. Samples were bottled and stored at –20°C until analysed. Total nitrogen was determined on a Sanplus Segmented Flow Analysis System from Skalar using method No. 155-316w/r issue 031898/MH/97203066.

Data analysis. The General Linear Models (GLM) procedure of the Statistical Analysis System (SAS Inc. 1990) was used to analyse the data.

Results and Discussion

Tree size. Significant r values of 0.54 and 0.83 were found between initial and final stem diameter and initial and final tree height, respectively (Table 1). The results clearly indicate that larger nursery trees grow stronger during the initial stages of development. In cool and cold production regions, coupled with a high frequency of wind, the initial tree growth is unsatisfactory, therefore planting large nursery trees may improve initial tree growth.

Topping height. Significant differences were obtained in stem diameter increase in 'Eureka' lemon, but there was no consistent pattern, which indicates that the root rot disease probably affected tree growth even before March 1999 (Table 2). In the case of 'Mihowase' Satsuma the stem diameter increase of all topped trees was significantly less than the untopped trees ($P = 0.0005$). A trend was observed with topping height, with the least increase in stem diameter with the most severe topping treatment.

Chemical and physical profile

Dry weight. Roots comprised approximately 22% of total dry mass, whilst the aboveground portions between 0 – 60 cm comprised 39 to 47% and above 60 cm, comprised 33 to 37% of total dry weight (Table 3).

Carbohydrates. Carbohydrate concentrations in the roots were generally higher compared to the aboveground portions (Table 4). Although, roots had high concentrations, it only comprised about one third the total carbohydrates of the tree.

The aboveground sections comprising about 78% of total dry weight (Table 3), contained more than two thirds of the total carbohydrates, of this 36 to 39% in the section of 0 - 60 cm, and 29 to 33% above 60 cm (Table 4).

Nitrogen. Concentrations in the roots and upper-most part of tree (100 cm +) were slightly higher compared to other aboveground parts (Table 4). Roots comprised 28 to 31% total nitrogen, aboveground sections between 0 and 60 cm, 23 to 35% and above 60 cm 37 to 46% (Table 4).

Our results generally showed that roots contained nearly one third or less of total reserves. This is in agreement with Boswell and Cole (1978) who found that citrus trees do not store large amount of carbohydrate in the root system. In contrast, nearly two thirds of the total carbohydrates were found in the roots of apple nursery trees (Steyn, 1998). Insufficient carbohydrate and nitrogen reserves are often implicated in poor establishment of nursery trees. Topping of nursery whip trees at 60 cm aboveground thus would result in a loss of 33 to 37% dry matter, 29 to 33% carbohydrates and 37 to 46% nitrogen. In a parallel study conducted in an attempt to investigate the effect of topping heights on initial growth, topping of nursery trees was found to reduce initial vegetative growth. It would therefore seem that retaining of the nursery-produced reserves would be advantageous to translate into quicker tree growth and consequently earlier yields.

Transplanting tall nursery whip trees imply that irrigation should be optimum from the day of planting since no adjustment to the root/shoot ratio is done at planting. Some type of tree support is usually required since the tall whip cannot support itself

The trees seemingly have to be established well before well before they will respond, possibly explaining the lack of significant results obtained with the first trial.

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Table 1. Pearson correlation coefficients between initial tree size (stem diameter and tree height) and final stem diameter and tree height of 'Eureka' lemon.

Correlations	PCC[x]	Sign. level[y]
Initial stem diameter vs. final stem diameter	0.54016	0.0001
Initial tree height vs. final tree height	0.82921	0.0001

[x]PCC – Peason's Correlation Coefficient

[y]Significance level

Table 2. Effect of topping height on stem diameter increase: 'Eureka' lemon on rough lemon rootstock and 'Mihowase' Satsuma on 'Carrizo' citrange rootstocks.

Topping height (cm aboveground)	Stem diameter (mm)		Stem diameter increase (mm)
	'Eureka' lemon		
	3/3/98	2/3/99	
80	8.52 a	22.52 b	14.00 b
120	9.06 a	26.91 a	17.85 a
Untopped	8.63 a	24.44 b	15.81 ab
LSD	0.76	2.19	2.07
Sign. level	0.3291	0.0010	0.0024
	'Mihowase' Satsuma		
	1/12/98	5/8/99	
60	5.07 a	9.41 c	4.34 c
80	4.98 a	9.91 cb	4.92 cb
100	5.02 a	11.13 b	6.11 b
120	5.14 a	10.61 cb	5.47 cb
Untopped	4.94 a	13.07 a	8.13 a
LSD	0.39	1.58	1.64
Sign. level	0.8706	0.0004	0.0005

Stem diameter measured at 5 cm above the bud union and 50 cm above ground of 'Eureka' lemon and 'Mihowase' Satsuma, respectively.

Table 3. Dry mass (g/tree) and % per part of tall nursery trees (1.4 –1.9 m) of 'Nules' Clementine on 'Carrizo' citrange and 'Eureka lemon' on rough lemon.

	Nules Clementine		Eureka lemon	
	Dry mass (g/tree)	%	Dry mass (g/tree)	%
Roots	16 b	20	21 b	24
Aboveground total	63	80	66	76
0 – 60 cm	37 a	47	34 a	39
60 - 100 cm	12 c	15	15 b	17
100 cm +	14 cb	18	17 b	20
Total tree	79	100	87	100
LSD (0.05)	4		5	
Sign. level	0.0001		0.0001	

Table 4. Carbohydrate (starch and soluble sugars) and total nitrogen reserve levels of different portions of 'Nules' Clementines and 'Eureka' lemon trees budded to 'Carrizo' citrange and rough lemon rootstocks, 1.4 -1.9 m tall whip trees.

	Carbohydrate reserves			Nitrogen reserves		
	mg/g	mg/tree	%	mg/g	mg/tree	%
			'Nules' Clementine			
Roots	113 a	1849 a	35	11 a	182 ab	28
Rootstock & scion (0 – 60 cm)	50 b	1860 a	36	6 c	230 a	35
Scion (60 – 100 cm)	61 b	719 b	14	9 b	102 c	16
Scion (100 cm +)	58 b	782 b	15	11 a	137 bc	21
Total		5210	100		651	100
LSD (0.05)	13	351		2	49	
Sign. level	0.0001	0.0001		0.0001	0.0001	
			'Eureka' lemon			
Roots	96 a	2053 b	28	14 a	296 a	31
Rootstock & scion (0 – 60 cm)	82 ab	2834 a	39	7 b	224 a	23
Scion (60 – 100 cm)	61 b	918 c	13	13 a	210 a	22
Scion (100 cm +)	86 a	1419 bc	20	14 a	230 a	24
Total		7224	100		960	100
LSD (0.05)	23	735		5	118	
Sign. level	0.0002	0.0002		0.0190	0.4489	

4. PAPER 3: PHYSICAL AND CHEMICAL MANIPULATION TO STIMULATE LATERAL BRANCHING OF SATSUMA TREES.

Abstract

Citrus nursery trees do not develop sylleptic shoots adequately in the nursery or in the field. However, even if it were possible the evergreen nature of citrus trees may preclude successful transplantation of such trees. When tall nursery whip trees are planted in the field, proximal buds do not readily sprout, with the apical sections invariable flushing first. Two trials in the Stellenbosch region on newly-planted (one or five months after planting) 'Mihowase' Satsuma trees were conducted to evaluate various branch induction techniques. Treatments consisted of girdling, Promalin® (GA_{4+7} + BA) and/or leaf removal and notching at 1000 or 2000 mg/*l* either to individual buds or the whole trees. In the second trial notching and girdling significantly increased the number and total length of lateral branches. In the first trial, on the very young trees, no significant results were obtained. In conclusion, the results of our studies evaluating physical and chemical side shoot induction suggest that notching is the most effective technique and should be evaluated further. The trees seemingly have to be established well before they will respond, possibly explaining the lack of significant results obtained with the first trial.

Introduction

The quality of nursery trees to be planted in the orchard is a major factor determining not only the rate at which the orchard becomes established, but also the level of productivity in the early years (Van Oosten, 1978). The use of well-branched nursery trees for orchard planting can shorten the time to commercial fruit production (Quinlan, 1978). Thus, by increasing the number of actively-growing shoots, allotted space is filled early, more leaf surface is available for photosynthetic activity, generally enhancing tree canopy development. The cost of establishment, especially at higher densities, necessitates quicker filling of the allotted space, and consequently earlier yields.

Unfortunately, citrus nursery trees do not branch adequately in the nursery or in the field. However, even if it were possible the evergreen nature of citrus trees may preclude successful transplantation of such trees. When tall nursery whip trees are planted in the field, proximal buds do not readily sprout, with the apical sections invariably flushing first. During subsequent flushes, lower lateral buds start to become active (Rabe, pers. comm.[3]). Conventionally, nursery whip trees are topped at 60 cm aboveground, allowing formation of scaffold branches between 40 to 60 cm aboveground (Tibshraeny, 1995). However, topping discourages early production by removing a lot of dry matter already produced in the nursery (Rabe, 1996).

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Also, the most apical branches assume a very upright growth habit, which suggests that it should have been left untopped from the outset.

Methods to stimulate early, lower lateral branches are needed when long whip trees are established. Notching, removing a thin section of the bark directly above the lateral bud, is a practice utilised in deciduous fruit trees to stimulate proleptic lateral branching (Greene and Autio, 1994). Shoots that develop from such notched buds have a narrow, sharper crotch angle (Greene and Miller, 1988). Promalin®, a commercial mixture of gibberellins (GA_{4+7}) and cytokinin (benzyl-adenine) induced proleptic lateral branching with wider crotch angles on one-year-old wood of young cherry and apple trees (Veinbrants and Miller, 1981; Williams and Billingsley 1970). Promalin® increased branching of apple trees, but significantly reduced tree height (Koen *et al.,* 1989; Quellete *et al.,* 1996). Removal of immature, apical leaves increases the number of branches in some apple cultivars, while removal of older leaves has less effect on sylleptic branching (Wertheim, 1978; Theron *et al.*, 1987; Popenoe and Barrit, 1988). By combining leaf removal and Promalin® it resulted in a more uniform distribution of laterals along the length of the central leader (Quellete *et al.*, 1996). No such information is available on citrus. Our objectives were thus to evaluate various branch-inducing techniques on newly-planted 'Mihowase' Satsuma to enhance initial growth and tree complexity.

Materials and Methods

Plant material. Two trials were conducted on newly-planted (1 month and 5 months old after planting) 'Mihowase' Satsuma trees budded to 'Carrizo' citrange rootstock,

spaced at 4 x 1.5 m and planted in the Stellenbosch region (34°S 19°E, ca. 119 m above sea level, mediterranean climate). Individual trees were selected for uniformity of size and vigour.

Treatments and statistical design. Both trials were laid out as randomised complete block designs with nine (Trial 1) and four (Trial 2) treatments replicated ten times using single tree plots.

The treatments of Trial 1 consisted of an untreated control (1), Promalin® (GA_{4+7} + BA) at 1000 or 2000 mg/*l* water plus Agral 90 at 0.2 m*l*/*l* as wetting agent either to individual buds or whole tree (2, 3, 4 and 5), leaf removal (6) and notching (7) (removal of a strip of bark 2 mm wide) and combination of leaf removal (8) or notching (9) with Promalin® at 1000 mg/*l* water applied to individual buds (every second bud from 50 cm aboveground). In Trial 2 the treatments consisted of an untreated control (1) notching (every second bud between 50 – 80 cm aboveground) (2), girdling at 70 cm aboveground (3) and Promalin® at 1000 mg/l water plus Agral 90 at 0.2 m*l*/*l* as wetting agent (4). Leaf removal was done by hand and notching was done by hacksaw blade above the bud (to every second bud 50 cm aboveground). This cut was approximately 5 mm above the bud and extended around about one-third of the circumference of the stem. Promalin® was applied with a small brush to individual buds. Treatments were applied on 26 November 1998 (Trial 1) and 01 April 1999 (Trial 2).

Data recorded. Stem diameter at 50 cm aboveground, tree height, and number and length of lateral branches were recorded.

Data analysis. The General Linear Model (GLM) procedures of the Statistical Analysis System (SAS Inc. 1990) was used to analyse the data.

Results and Discussion

No significant increase in number of laterals relative to the control was obtained in Trial 1 (Table 1). The trees were only established one month prior to the treatment and therefore probably not well established to respond to the treatments. Notching significantly increased the total number ($P = 0.0001$) (approximately 5) and total length (≥16 cm) of lateral shoots ($P = 0.0001$) (Table 2). However, there were no significant trends relative to stem diameter and tree height. Girdling induced lateral branches, which were not satisfactory in length (≤5 cm) (Table 2) and were formed just within the girdled area. Maybe several girdles along the trunk axis could result in satisfactory lateral branch induction. Promalin® did not stimulate lateral branching.

When tall nursery whip trees are planted in the field, proximal buds do not readily sprout, with the apical sections invariable flushing first. Notching altered the natural growth response by increasing budbreak. Notching and girdling, thus stimulates lateral branching by interrupting the downward movement of auxin (Tamas, 1987). However, lateral branches stimulated were observed to have narrow crotch angles, which confirms earlier findings on deciduous trees (Greene and Miller, 1988). This is not really regarded as a problem in citrus. Although, girdling induced a significant number of lateral branches, the branches were not well distributed along the stem.

In conclusion, these studies on evaluating physical and chemical side shoot induction suggest that notching is the most effective technique and should be further evaluated.

The trees seemingly have to be established well before well before they will respond, possibly explaining the lack of significant results obtained with the first trial.

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Table 1. Effect of Promalin®, leaf removal and notching and combination thereof on lateral branch induction of 1-month-old 'Mihowase' Satsuma trees (applications made on 26 November 1998).

Treatment	Conc. (mg/*l*)	Stem diameter (mm)[z]			Tree height (cm)			No. of lateral shoots[y]	Total shoot length (cm)
		26/11	30/07	Increase	26/11	26/07	Increase		
Control		4.98 ab	9.46 bcd	4.48 bcd	131 bcd	159 abc	28 ab	5.1 ba	14.62 c
Promalin (applied to the whole tree)	1000	4.75 bc	9.51 bcd	4.76 bcd	140 a	152 abc	2 b	4.50 abc	21.84 bc
Promalin (applied to the individual buds)	1000	4.85 abc	10.42 abc	5.57 abc	136 abc	165 ab	29 a	2.10 d	18.78 bc
Promalin (applied to the whole tree)	2000	4.69 bc	10.69 ab	6.00 ab	139 ab	167 a	28 ab	3.10 d	32.24 a
Promalin (applied to the individual buds)	2000	4.93 ab	8.70 d	3.77 d	131 cd	153 abc	22 ab	4.30 abc	23.59 b
Leaf removal (every 2nd bud)		5.09 ab	9.01 cd	3.92 cd	139 cd	160 abc	21 ab	1.80 d	13.62 c
Notching (every 2nd bud)[x]		4.88 c	10.22 bcd	5.34 abcd	133 abcd	147 c	14 b	6.00 a	19.52
Leaf removal plus Promalin	1000	4.41 c	8.79 cd	4.37 bcd	127 d	151 bc	24 ab	4.10 bc	20.24 bc
Notching plus Promalin	1000	5.27 a	12.08 a	6.81 a	135 abc	151 bc	16 b	5.20 ab	18.06 bc
LSD (0.05)		0.52	1.69	1.69	7	14	14	1.88	8.25
Sign. level		0.0938	0.0051	0.0051	0.0114	0.1718	0.1718	0.0002	0.0010

[x]Notching was done on every second bud 50 cm aboveground level. [y]Lateral shoots were counted between 50 and ±120 cm above ground. [z]Stem diamter measured at 50 cm above ground .

Table 2. Effect of notching, girdling, and Promalin® on branch induction of 5-month-old of 'Mihowase' Satsuma trees (applications made on 1 April 1999).

Treatment	Stem diameter (mm)[w]			Tree height (cm)			No. of lateral shoots[z]	Total shoot length (cm)
	01/04	29/07	Increase	01/04	29/07	Increase		
Control	9.58 a	12.95 a	3.37 a	158 a	176 a	18 a	0 c	0 c
Notching (every 2nd bud)[x]	9.29 a	13.41 a	4.12 a	166 a	179 0a	13 a	5 a	17 a
Girdling (at 70 cm above ground)[y]	8.93 a	10.81 b	1.88 b	164 a	178 a	4 a	2 b	5 b
Promalin (1000 mg/*l*)	9.87 a	14.11 a	4.24 a	163 a	183 a	20 a	0 c	0 c
LSD	1.09	1.62	1.06	12	14	13	1	5
Sign. level	0.3446	0.0019	0.0003	0.6051	0.8032	0.6082	0.0001	0.0001

[x]Notching was done on every second bud between 50 – 80 cm aboveground level.
[y]Girdling was at 70 cm above ground level.
[z]Lateral shoots were counted between 50 – 80 cm above ground level.
[w]Stem diameter measured at 50 cm aboveground level.

5. PAPER 4: EFFECTS OF EARLY WINTER GIBBERELLIC ACID AND MINERAL OIL APPLICATIONS ON FLOWERING OF YOUNG NON-BEARING CITRUS TREES.

Abstract

In young, non-bearing trees a lot of energy is invested in the flowering process, which results in an inhibition of vegetative growth. Gibberellic acid (GA_3) and heavy mineral oil (Bac-oil) treatments, either individually or in combination, applied during early winter were evaluated as a means to reduce flowering levels in young non-bearing citrus trees. Five trials were conducted over four seasons. GA_3 and mineral oil either separately or in combination were sprayed from April to July to the whole tree. GA_3 and mineral oil either individually or in combination applied late in May to late June markedly reduced flowering. GA_3 application in mid April had a minimal effect on flowering levels. Peak responses to GA_3 coincided with a significant reduction in bud sprouting. Although optimum application time proved to be around May to July, this time is likely to vary from year to year depending on the influence of the prevailing temperature and other climatic conditions. The winter application of GA_3, specifically, and possibly in combination with mineral oil to inhibit flowering and enhance early vegetative growth may be utilised commercially. However, response may vary dramatically from season to season.

Introduction

In young, non-bearing trees a lot of energy is invested in the flowering process, which results in the inhibition of vegetative growth, especially, in cool and cold climatic regions. Flowers on young trees can also set heavily and the presence of such fruits leads to competition for carbohydrate reserves, thus reducing overall vegetative growth and canopy increase.

GA_3 and mineral oils are known to inhibit flowering in citrus when applied in winter, but the mechanism of inhibition is not fully understood. Addition of mineral oil to GA_3 enhanced the effectiveness of GA_3 (Moss, 1974). An interference with the flower initiation process has been suggested (Monselise and Halevy, 1964; Moss, 1970; 1974), since GA_3 is most effective when applied during the winter rest period, at the time when some flowering factor is translocated from the leaves to the buds (Furr and Armstrong, 1956) or when applied during a period of water stress, which usually induces flowering in lemon (Nir *et al.*, 1972).

The timing of exogenously applied GA_3 treatments to buds is important in terms of its inhibitory effects. The best result occurs at the onset of bud initiation, but it decreases after differentiation has begun (Guardiola, *et al.*, 1977). Iwahori and Oohata (1981) confirmed this by correlating the inhibition of inflorescence development by GA_3 with the morphological development of the buds. Lord and Eckard (1987) determined that GA_3 application could not cause reversion of developing inflorescence buds to a vegetative state once sepals were initiated. However, late applications of GA_3, once

macroscopic bud sprouting occurs when flower differentiation is microscopically detectable can be effective (Nir *et al.*, 1972; Goldschmidt and Monselise, 1972).

GA_3 sprays generally reduce generative shoot production while the number of developing vegetative shoots remains similar to the control (Monselise and Halevy 1964; Goldschmidt and Monselise, 1972). These findings are in contrast to other reports (Nir *et al.*, 1972; Guardiola *et al.*, 1977) who found that a marked reduction in flower number result in an increase in vegetative shoots. These authors also suggested that the increase in the number of vegetative shoots is brought by reversion of generative buds. Southwick and Davenport (1987), by contrast, reported substantial inhibition of both flowering and vegetative shoots of when GA_3 was applied on 'Tahiti' lime.

The present study was undertaken to determine the optimum application time for GA_3 and/or mineral oil to inhibit flower initiation and flowering levels to enhance vegetative growth.

Materials and Methods

Plant material. Five trials, two on 'Mihowase' Satsuma and three on 'Nules' Clementine were conducted over four seasons in Western Cape area of South Africa (34°S 19°E, ca. 100 m above sea level, mediterranean climate). Individual trees were selected for uniformity of size and vigour.

GA_3 and mineral oil, either separately or in combination were sprayed from April to July to the whole tree using a spray application until run-off. Treatments and statistical design for all trials are presented in Table 1.

Data recorded. Two healthy, one-year-old branches were tagged per tree at two opposite sides of the tree at the time of treatment. In October, during the flowering period the number of nodes, newly-sprouted buds, flowers and newly-emerged leaves were counted on pre-tagged branches. Sprouting percentage was calculated based on the number of sprouted shoots per total node number.

Data analysis. The General Linear Models (GLM) procedure of the Statistical Analysis System (SAS Inc. 1990) was used to analyse the data.

Results

Effects on flowering. The application of GA_3 and mineral oil individually or in combination anytime from late May up to late July significantly reduced flowering in 'Mihowase' Satsuma (Trials C and D) and 'Nules' Clementine (Trial E only) ($P = 0.0001$, $P = 0.0001$ and $P = 0.0196$; Tables 2, 3 and 4 respectively). Non-significant results were obtained with other trials using 'Nules' Clementine (Trial A and B) ($P = 0.5576$ and 0.3431; Tables 5 and 6 respectively), although flowering was reduced with GA_3 application in June.

Effects on sprouting. The most reduction in flowering was paralleled by a significant reduction in bud sprouting ($P = 0.0002$ and $P = 0.0001$) (Tables 2 and 3). The number

of new leaves increased slightly (Tables 3 and 4) and sometimes significantly, especially with the late application on June 23 (Table 2).

When sprouting percentage is used as a covariate for flowering, the significant difference for flowering between treatments still existed, but was sometimes slightly reduced ($P = 0.0062$ and $P = 0.0211$; Tables 4 and 6). The levels of flowering were thus partially as a result of the number of nodes which sprouted and mainly as a direct effect of treatments on reducing sprouting incidence.

Discussion

In citrus, flower induction is believed to start during the early winter (June/July; S.H.) in subtropical regions (Monselise and Halevy, 1964), while flower initiation occurs during budbreak (Ayalon and Monselise, 1960; Iwahori and Oohata, 1981; Lord and Eckard, 1985). Guardiola (1981) believe that some stages of flower formation even starts as early as March (S.H.).

GA_3 and/or mineral oil were generally more effective at 50 mg/*l* and/or 3%, respectively. Our results further indicate that, the period of effective inhibition of flowering occurred during the period extending from late May to late July where GA_3 and/or mineral oil application was somewhat effective in reducing flowering levels. This is timed with the beginning of the translocation of an unknown flowering signal from leaves to the buds (Sánchez-Capuchino and Casanova, 1973). Application in mid April is too early for satisfactory results. Monselise and Halevy (1964) and Moss (1970), suggest that GA_3 interfere with the flower initiation process. Thus, the

greatest effects of flower inhibiting treatments should be at the onset of bud initiation (Guardiola *et al.*, 1977). Our results therefore indirectly confirm that flower bud initiation in 'Nules' Clementine and 'Mihowase' Satsuma take place around late May to late July under Western Cape conditions.

Guardiola *et al.* (1977) reported that for 'Navelate' and 'Washington' navel orange the main effect of GA_3 on flowering resides in the inhibition of bud sprouting, rather than on flower induction. Our results are consistent herewith, since the best treatment in reducing flowering levels coincided with a significant reduction in bud sprouting, although a direct effect on flower initiation cannot be excluded (Tables 3, 4 and 5). Where flowering was almost completely inhibited, bud sprouting was reduced significantly (Tables 4 and 5). Thus, the possibility of inhibition of both flowering and vegetative shoots, as demonstrated by Southwick and Davenport (1987) on 'Tahiti' lime, is likely. However, our results cannot be explained completely through inhibition of bud sprouting, since GA_3 and/or mineral oil in combination also reduced flowering significantly without significantly inhibiting bud sprouting (Tables 4 and 6).

Although GA_3 and/or mineral oil application during winter effectively decrease flowering. Mineral oil generally does not seem to be very effective (Tables 3, 4, 5 and 6). It is unlikely that the addition of mineral oil to GA_3 enhanced the effectiveness of GA_3 under our conditions as has been reported by Moss (1974). GA_3 and mineral oil did not significantly reduced flowering as compared to GA_3 alone, although, sometimes, good results were obtained with GA_3 and mineral oil, especially with May to mid June applications (Tables 5 and 6).

Although optimum application time seems to be during the late May to late July period, this time is likely to vary somewhat from year to year. Also, lack of a consistent significant reduction in flowering levels may prevent the commercial usage of GA_3 and/or mineral oil for this purpose on young trees.

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Table 1. Treatments and statistical design of GA_3 and mineral oil trials (A, B, C).

	Trial A	Trial B	Trial C
Cultivar	'Nules' Clementine	'Nules' Clementine	'Mihowase' Satsuma
Planting date	1993	1994	1997
Rootstock	'Carrizo' citrange	'Carrizo' citrange	'Carrizo' citrange
Tree spacing (m)	4.0 x 1.0	4.5 x 2.0	4.5 x 1.5
Tree age (year)	1	3	1
Treatments	(1) non-sprayed control; GA_3 at 25 mg/*l* water, (2) 12 April, (3) 12 May, (4) 13 June, (5) 12 July and (6) 12 April + 12 May. Agral 90 at 0.15 m*l*/*l* was used as wetting agent. Trees were sprayed until run-off.	(1) non-sprayed control; (2) GA_3; (3) mineral oil; and (4) GA_3 + mineral oil. GA_3 and mineral oil concentrations were 20 mg/*l* water and 1.5% water, respectively, applied on 23 June. Agral 90 at 0.15 m*l*/*l* was used as wetting agent. Trees were sprayed until run-off.	(1) non-sprayed control; GA_3 on (2) June 2, (3) June 23; (4) mineral oil on June 2 and (5) June 23; GA_3 + mineral oil on (6) June 2 and (7) June 23. GA_3 and mineral oil concentrations were 50 mg/*l* water and 3% water, respectively. Agral 90 at 0.2 m*l*/*l* was used as wetting agent on treatment 2 and 3 only. Trees were sprayed until run-off.
Statistical (Randomised complete block design	10 replicates of 2-tree plots.	4 replicates of 4-tree plots.	10 replicates of 2-tree plots.

Table 1 (continue)

Trials (D and E)

Cultivar	'Mihowase' Satsuma (Trial D) 'Nules' Clementine(Trial E)
Planting date	1997 (Trial D) and 1996 (Trial E)
Rootstock	'Troyer' citrange
Tree spacing	4.5 x 2.0 (Trial D) and 5.0 x 2.5 (Trial E)
Tree age (year)	2 (Trial D) and 3 (Trial E)
Treatments	(1) non-sprayed control; (2) GA_3 (2) on May 21, (3) June 4, (4) June 17, (5) July 5, (6) July 21; mineral oil (7) on May 21, (8) June 4, (9) June 17, (10) July 5, (11) July 21; (12) GA_3 and mineral oil on May 21, (13) June 4, (14) June 17, (15) July 5, (16) June 21. GA_3 and mineral oil concentrations were 50 mg/*l* water and 3% water, respectively. Agral 90 at 0.2 m*l*/*l* was used as wetting agent on treatments 2, 3, 4, 5, and 6. Trees were sprayed until run-off.
Statistical design Randomised complete block design	10 replicates of single tree plots.

Table 2. Effect of GA_3 (50 mg/*l*) and/or mineral oil (3%) applied at different times on bud sprouting, flowering, and new leaves on tagged brances of 1-year-old 'Mihowase' Satsuma during 1998.

Treatments	Sprouting %[y]	Average No. of flowers	Average No. of new leaves
Unsprayed control	56.9 a	16.4a	37.6 c
2 June, GA_3	37.2 d	0.2 d	44.9 bc
23 June, GA_3	55.3 ab	5.3 bcd	60.2 ab
2 June, Mineral oil	55.2 ab	11.9 ab	45.0 bc
23 June, Mineral oil	47.78 bc	7.3 bc	37.9 c
2 June, GA_3 and Mineral oil	44.2 dc	3.8 cd	49.0 bc
23 June, GA_3 and Mineral oil	50.1 abc	2.40 cd	73.8 a
LSD (0.05)	8.6	6.7	21.5

Sign. Level	**Pr>f**	**Pr>f**	**Pr>f**
Treatment	0.0002	0.0001	0.0145
Covariate analysis:			
Sprouting Percentage		0.0001	
Treatment		0.0062	

[y]Sprouting percentage was calculated based on the number of sprouted buds per number of nodes.

Table 3. Effect of GA_3 (50 mg/*l*) and/or mineral oil (3%) applied at different times on bud sprouting, and flowering on tagged shoots of 2-year-old 'Mihowase' Satsuma during 1999.

Treatments	Sprouting %[y]	Average No. of flowers
Unsprayed control	60. 8 a	7.2 a
21 May, GA_3	49.2 bc	5.7 ab
4 June, GA_3	42.7 bc	2.3 cde
17 June, GA_3	40.1 bc	0.7 de
5 July, GA_3	45.8 ab	1.1 cde
21 July, GA_3	46.1 ab	0.7 de
21 May, Mineral oil	51. 4 ab	1.6 cde
4 June, Mineral oil	47. 6 ab	3.1 bcd
17 June, Mineral oil	53.6 ab	3.4 bc
5 July, Mineral oil	55.1 ab	0.9 cde
21 July, Mineral oil	54.7 ab	1.7 cde
21 May, GA_3 and Mineral oil	21.3 d	0.0 e
4 June, GA_3 and Mineral oil	27.3 cd	0.1 e
17 June, GA_3 and Mineral oil	27.8 cd	0.45 e
5 July, GA_3 and Mineral oil	42. 4 bc	2.0 cde
21 July, GA_3 and Mineral oil	50. 3 ab	1.0 cde
LSD (0.05)	15.9	2.6
Sign. Level	**Pr>f**	**Pr>f**
Treatment	0.0001	0.0001
Covariate analysis:		
Sprouting Percentage		0.0001
Treatment		0.0001

[y]Sprouting percentage was calculated based on the number of sprouted buds per number of nodes.

Table 4. Effect of GA_3 (50 mg/*l*) and/or mineral oil (3%) applied at different times on bud sprouting, and flowering on tagged branches of 2-year-old 'Nules' Clementine during 1999.

Treatments	Sprouting %[y]	Average No. of flowers
Unsprayed control	58.6 abcd	12.5 a
21 May, GA_3	67.1 a	7.1 ab
4 June, GA_3	56.7 abcd	0.8 c
17 June, GA_3	64.2 abc	2.6 bc
5 July, GA_3	47.2 cd	4.1 bc
21 July, GA_3	65.0 ab	4.8 bc
21 May, Mineral oil	59.6 abcd	4.8 bc
4 June, Mineral oil	51.9 abcd	6.1 bc
17 June, Mineral oil	64.3 abc	1.0 c
5 July, Mineral oil	60.9 abcd	1.5 bc
21 July, Mineral oil	66.1 ab	3.7 bc
21 May, GA_3 and Mineral oil	49.6 bcd	4.1 bc
4 June, GA_3 and Mineral oil	44.9 d	1.9 bc
17 June, GA_3 and Mineral oil	59.2 abcd	1.4 bc
5 July, GA_3 and Mineral oil	65.9 ab	3.9 bc
21 July, GA_3 and Mineral oil	56.8 abcd	5.2 bc
LSD (0.05)	17.5	7.2 ab
Sign. Level	**Pr>f**	**Pr>f**
Treatment	0.2659	0.0196
Covariate analysis:		
Sprouting Percentage		0.0196
Treatment		0.0211

[y]Sprouting percentage was calculated based on the number of sprouted buds per number of nodes.

Table 5. Effect of GA_3 (25 mg/*l*) applied at different times on the flower number and new leaves on single tagged branches of 1-year-old 'Nules' Clementine during 1994.

Treatments	Average No. of flowers	Average No. of new leaves
Control	5.8 a	67.9 ab
12 April	5.5 a	64.4 b
12 May	5.8 a	71.4 ab
13 June	2.9 a	73.3 ab
12 July	4.6 a	65.7.b
12 April + 12 May	4.8 a	77.7.a
LSD (0.05)	3.5	11.3
Sign. level	**Pr>f**	**Pr>f**
Treatment	0.5576	0.1791

Table 6. Effect of GA_3 (20 mg/*l*) and mineral oil (1.5%) applied on 23 June bud sprouting, flowering, and new leaves on tagged branches of 3-year-old 'Nules' Clementine during 1997.

Treatments	Sprouting %[y]	Average No. of flowers	Average No. of new leaves
Control	53.5 ab	72.3 a	186.0 ab
GA_3	37.9 b	22.8 a	134.5 b
Mineral oil	50.7 ab	33.0 a	182.3 ab
GA_3 and Mineral oil	71.9 a	58.8 a	58.5 a
LSD (0.05)	28.5	66.5	110.0
Sign. level	**Pr>f**	**Pr>f**	**Pr>f**
Treatment	0.1275	0.3431	0.1359
Covariete Analysis			
Sprouting Percentage		0.4027	
Treatment		0.4457	

[y]Sprouting percentage was calculated based on the number of sprouted buds per number of nodes.

6. PAPER 5. EARLY CHEMICAL FRUIT THINNING ON YOUNG NON-BEARING CITRUS TREES TO ENHANCE VEGETATIVE GROWTH.

Abstract

Young, non-bearing citrus trees can be precocious, resulting in excess flowering and initial fruit set, leading to reduced vegetative growth. Effect of time of application and concentration of 2,4-dichlorophenoxypropionic acid (2,4-DP) and 1-naphthaleneacetic acid (NAA) as potential fruit thinning agents to reduce fruit set soon after flowering and enhance vegetative growth were evaluated over a two year period. 2,4-DP at 150 and 300 mg/*l* and NAA at 200 and 400 mg/*l* were sprayed on 2- and 4-year-old 'Mihowase' Satsuma, as well as on 2-year-old 'Marisol', 'Nules' and 'Oroval' Clementine trees at two times (late October and early November). The higher concentration of 2,4-DP and NAA generally did not result in increased fruit abscission in the first year. However, in the case of 2,4-DP the higher concentration resulted in stronger thinning in the second year. The possibility of using 2,4-DP and NAA as potential fruit thinning agents to reduce fruit set on young non-bearing trees should be explored in additional trials.

Introduction

In young non-bearing trees a lot of energy is invested in the flowering process, which results in the inhibition of vegetative growth, especially, in cool and cold climatic regions. Flowers on young trees can also set heavily and the presence of such fruit leads to competition for carbohydrate reserves, thus reducing overall vegetative growth and canopy increase.

Cameron (1932) and Maggs (1963) reported an inverse relationship between vegetative growth and fruiting of orange and apple trees. Maggs (1963) and Avery (1969) further found that fruiting decreased the growth of the root system more than the aerial parts of potted 'Worcester Pearmain' apple trees compared with non-fruiting ones. In citrus trees with a heavy crop load (e.g. during an 'on' year of alternate-bearing cultivars) root growth is checked, presumably due to a depletion of carbohydrates (Goldschmidt and Golomb, 1982).

Reduction in fruit number has been reported to enhance vegetative growth. Deblossoming experiments by Maggs (1963) on two-year-old apple trees showed greater dry-weight, increased leaf size, leaf number, and stem diameter in deblossomed than in fruiting trees. Jones *et al.* (1974) found an increase in vegetative growth when all fruits were removed three months after bloom in 'Valencia' orange trees.

Thinning of fruitlets on young citrus is usually done by hand once practically possible. This is not only labour-intensive, but also done later than desired. 1-

naphthalene acetic acid (NAA) has been widely studied as a material for thinning citrus. Iwahori and Oohata (1976) found that spraying NAA at 300 mg/*l* 25 days after full bloom proved satisfactory for thinning 'Satsuma' mandarin. Rabe *et al.* (1995) in studying fruit size improvement of 'Clementine', reported that an application of 100 mg/*l* 2,4-dichlorophenoxypropionic acid (2,4-DP) on small fruits early in December (S.H.) resulted in more thinning of fruitlets.

Work done by Wheaton (1981), clearly demonstrated the importance of timing of NAA and other thinning materials on 'Dancy' tangerines and 'Murcotts'. He found that NAA and other thinning materials were effective in May (period of natural fruit drop), with diminished activity early June and no effect when applied in mid-June. By contrast, Ortolá *et al.* (1991) found that application of NAA at 200 mg/*l* late in June (period coinciding with the end of natural fruit drop) resulted in better thinning in 'Satsuma' mandarin. The timing difference in effectivity would be due to climatic differences, i.e. in hotter regions early sprays would be required for the best effect.

The aim of this study was to evaluate the effects of time of application and concentration of 2,4-DP and NAA as potential fruit thinning agents to reduce fruit set soon after flowering.

Materials and Methods

Plant material. Five trials, two on 'Mihowase' Satsuma and three on 'Clementine selections, viz. 'Marisol', 'Nules' and 'Oroval' were conducted over a two year period

in the Western Cape area of South Africa (34°S 19°E, ca. 100 m above sea level, mediterranean climate). Trees on 'Carrizo' citrange rootstock were 2 and 4 years old, spaced at 4.0 x 1.5 - 2.0 m. Individual trees were selected for uniformity of size, vigour and flowering intensity.

Treatment and statistical design. The treatments consisted of: non-sprayed control, 2,4-DP at two concentrations (150 mg/*l* water or 300 mg/*l* water) applied early or late, NAA at two concentrations 200 mg/*l* water or 400 mg/l water, applied early or late. The early treatments were applied late in October (just after blossom) and the late treatments were applied early in November (approx. 14 days later). Agral 90 at 1ml/*l* was used as a wetting agent. The spray solution was applied as a full cover spray until run-off to the entire tree using a handgun sprayer mounted on a light truck (4-year-old trees received approx. 3 *l* and 2-year-old trees received approx. 1.5 *l* per tree). The trials were conducted at three different sites over two consecutive years, using different trees. The treatments were replicated 10 times in a randomised complete block design using single tree plots, always leaving a border tree between treatments.

Data recorded. The diameter of 100 randomly selected fruitlets was measured at the time of the treatments (Table 1). 'Nules' and 'Oroval' Clementine flowers open continually over an extendend period. The population of fruitlets measured may thus have differed somewhat, accounting for the very small difference in fruit size between the two treatment dates.

On the first application date two shoots per tree were tagged on two opposite sides on the periphery of the tree canopy. Initial fruit number and the subsequent number of retained fruitlets per shoot were counted at two-week intervals. Fruit drop was expressed as a percentage of the initial fruit number on the shoots. At the end of the physiological fruit drop period all the remaining fruitlets on the entire tree were harvested and counted.

Data analysis. The General Linear Models (GLM) procedure of the Statistical Analysis System (SAS Inc. 1990) was used to analyse the data.

Results and Discussion

Average fruit sizes were around 5 mm in diameter for 'Clementine' selections and 6 mm and 10 mm or greater in diameter for 'Mihowase' Satsuma at the time of the treatments (Table 1).

Application of both 2,4-DP and NAA during the natural fruit drop significantly reduced the final number of fruits harvested (Tables 2 to 6), confirming other reports (Hield and Hilgeman, 1969; Hirose, 1981; Wheaton, 1981; Rabe *et al.*, 1995). However, this effect depends on the developmental stage of the fruitlets at the time of treatment. Generally, application of 2,4-DP and NAA (except that of NAA on 'Mihowase'; Table 5) when the mean fruitlets size is around 5 mm in diameter resulted in heavier thinning (Tables 3, 4, 5 and 6). In fact, where treatments were applied two weeks later, effectivity was mostly decreased significantly (Tables 2 to 6). Thus, the sooner treatments are applied after flowering more abscission resulted.

This reduction in fruit numbers was due to the enhancement of the natural fruit drop by application of 2,4-DP and NAA (Fig. 1 and 2), regardless of spray concentration or timing.

The 2,4-DP was generally more effective at higher concentration (300 mg/*l*) in 1998 (Tables 5 and 6) ($P = 0.0011$ and $P = 0.0204$) and NAA was effective at either 200 mg/*l* or 400 mg/*l* (Tables 2 to 6) ($P<0.005$). However, high concentrations of 2,4-DP caused some leaf curling in 'Mihowase', typical of auxin-induced damage.

Two periods of flower or natural fruit abscission occurs during and within a few weeks of bloom or fruit set (Davies, 1986). These periods may or may not be well separated depending on the time of bloom and prevailing weather conditions. The first period of abscission involves flowers and very young fruit (Wheaton and Stewart, 1973). The second period of abscission takes place later and is commonly referred to as November and December drop ('June drop') and occurrs in November. 2,4-DP and NAA applied prior to the 'June drop' is thus effective as a fruit thinning agent by enhancing and accelerating the natural drop period pattern.

In conclusion, the results of our studies evaluating 2,4-DP and NAA over a period of two seasons, indicated that both chemicals (2,4-DP at 300 mg/*l*) and NAA at 200 mg/*l*) applied soon after flowering (fruitlet size <5 mm) was effective in fruitlet thinning. Variability in the amount of thinning was observed, but 2,4-DP was usually more consistent and effective than NAA. Unfortunately we did not measure the response in vegetative growth to determine whether this reduction in crop load effectively advanced vegetative growth.

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Table 1: Average fruit sizes at different application dates of 2,4-DP and NAA.

DATE	FRUIT SIZE (mm)		
	Year 1 (1997)		
	'Mihowase' Satsuma	'Marisol' Clementine	' Nules' Clementine
21/10; early*	11.25	5.25	4.24
10/11; late**	22.33	7.37	4.95
	Year 2 (1998)		
	'Mihowase' Satsuma	'Oroval' Clementine	
26/10, early*	5.97	4.26	
11/11, late**	9.50	4.97	

* early: October application just after bloom.

**late: November application approximately 14 days later than early application.

Table 2. Fruit thinning effect of 2,4-DP (150 & 300 mg/*l*) and NAA (200 & 400 mg/*l*) applied 21 October and 10 November 1997 on 'Mihowase' Satsuma. Fruits were harvested on 22 December 1997.

Chemical applied	Date	Conc. (mg/*l*)	No. of fruit/ tree	% of control
Control			208 a	100
2,4-DP	21 Oct.	150	137 cd	66
2,4-DP	21 Oct.	300	140 cd	67
2,4-DP	10 Nov.	150	173 abc	83
2,4-DP	10 Nov.	300	172 cb	83
NAA	21 Oct.	200	132 d	63
NAA	21 Oct.	400	132 d	63
NAA	10 Nov.	200	178 ab	86
NAA	10 Nov.	400	152 bcd	73
LSD (0.05)			36	

Source	Pr>f
TRT	0.0004
Contrast	
Control vs. TRT	0.0001
2,4-DP vs. NAA	0.4411
Time 1 vs. Time 2	0.0004
Chemical vs. Time	0.9694
2,4-DP Concentration	0.9506
NAA Concentration	0.3085
2,4-DP Concentration x time	0.8709
NAA Concentration x time	0.3122

Table 3. Fruit thinning effect of 2,4-DP (150 & 300 mg/l) and NAA (200 & 400 mg/l) applied on 24 October and 13 November 1997 on 'Nules' Clementine. Fruits were harvested on 12 January 1998.

Chemical applied	Date	Conc. (mg/*l*)	No. of fruit/ tree	% of control
Control			42 a	100
2,4-DP	24 Oct.	150	14 b	33
2,4-DP	24 Oct.	300	5 b	12
2,4-DP	13 Nov.	150	39 ab	93
2,4-DP	13 Nov.	300	37 ab	88
NAA	24 Oct.	200	45 a	100
NAA	24 Oct.	400	24 ab	57
NAA	13 Nov.	200	20 ab	47
NAA	13 Nov.	400	48 a	100
LSD (0.05)			38	
Source			Pr>f	
TRT			0.2806	
Contrasts				
Control vs. TRT			0.3616	
2,4-DP vs. NAA			0.2895	
Time 1 vs. Time 2			0.1230	
Chemical x Time			0.1230	
2,4-DP Concentration			0.6768	
NAA Concentration			0.8348	
2,4-DP Concentration x Time			0.7810	
NAA Concentration x Time			0.0756	

Table 4. Fruit thinning effect of 2,4-DP (150 & 300 mg/*l*) and NAA (200 & 400 mg/*l*) applied on 24 October and 13 November 1997 on 'Marisol' Clementine. Fruit were harvested on 13 January 1998.

Chemical applied	Date	Conc. (mg/*l*)	No. of fruit/ tree	% of control
Control			110 a	100
2,4-DP	24 Oct.	150	22 cd	20
2,4-DP	24 Oct.	300	20 d	18
2,4-DP	13 Nov.	150	73 ab	66
2,4-DP	13 Nov.	300	64 bcd	58
NAA	24 Oct.	200	36 bcd	33
NAA	24 Oct.	400	20 d	18
NAA	13 Nov.	200	63 bcd	57
NAA	13 Nov.	400	68 abc	61
LSD (0.05)			48	

Source	Pr>f
TRT	0.0024
Contrasts	
Control vs.TRT	0.0005
2,4-DP vs. NAA	0.8329
Time 1 vs. Time 2	0.0007
Chemical x Time	0.6595
2,4-DP Concentration	0.7341
NAA Concentration	0.7363
2,4-DP Concentration x Time	0.8200
NAA Concentration x Time	0.5393

Table 5. Fruit thinning effect of 2,4-DP (150 & 300 mg/*l*) NAA (200 & 400 mg/*l*) applied 26 October and 11 November 1998 on 'Mihowase' Satsuma. Fruits were harvested on 22 December 1998.

Chemical applied	Date	Conc. (mg/*l*)	% Fruit retention/shoot Start	mid	end	No. of fruit/ tree	% of Control
Control			100	37	21	205 a	100
2,4-DP	26 Oct.	150	100	20	8	131 bc	64
2,4-DP	26 Oct.	300	100	39	7	74 d	36
2,4-DP	11 Nov.	150	100	41	15	122 c	60
2,4-DP	11 Nov.	300	100	45	6	79 d	39
NAA	26 Oct.	200	100	25	15	178 a	87
NAA	26 Oct.	400	100	31	18	167 bc	81
NAA	11 Nov.	200	100	26	10	92 cd	45
NAA	11 Nov.	400	100	34	11	98 cd	49
LSD (0.05)						41	

Source	Pr>f
TRT	0.0001
Contrast	
Control vs. TRT	0.0001
2,4-DP vs. NAA	0.0028
Time 1 vs. Time 2	0.0005
Chemical vs. Time	0.0005
2,4-DP Concentration	0.0011
NAA Concentration	0.8807
2,4-DP Concentration x time	0.6261
NAA Concentration x time	0.5718

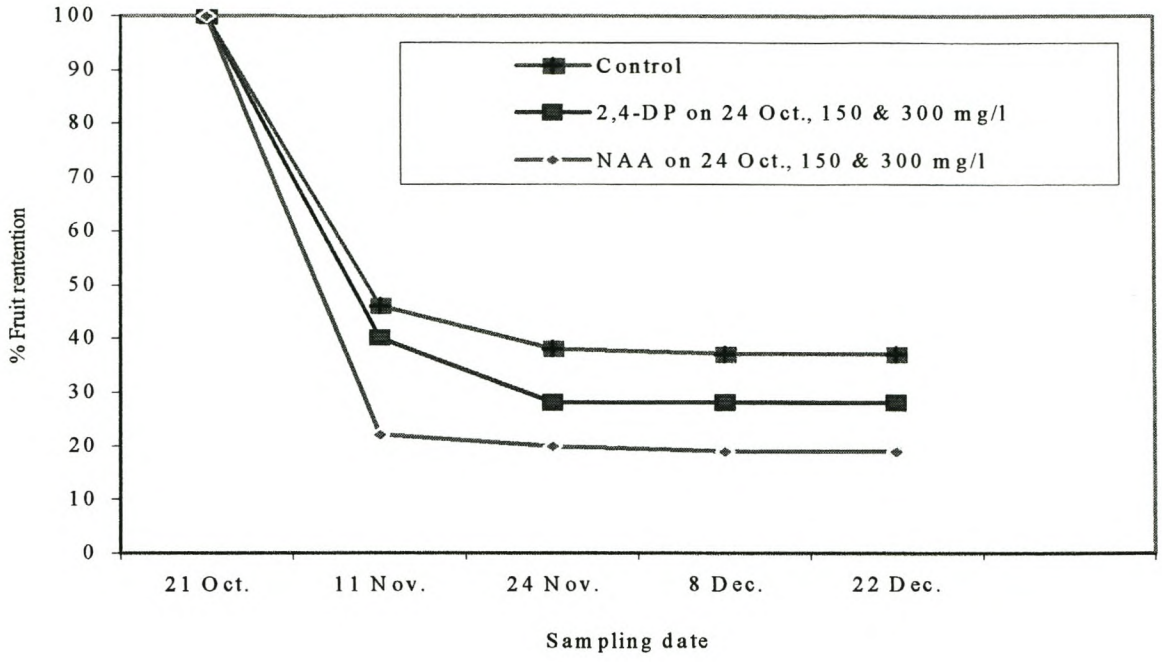
Table 6. Fruit thinning effect of 2,4-DP (150 & 300 mg/*l*) and NAA (200 & 400 mg/*l*) applied 26 October and 11 November 1998 on 'Oroval' Clementine. Fruit were harvested on 21 January 1999.

			% Fruit retention/shoot				
			start	mid	end		
Chemical applied	Date	Conc. (mg/*l*)				No. of fruit/ tree	% of control
Control			100	69	16	54 a	100
2,4-DP	26 Oct.	150	100	55	16	41 ab	76
2,4-DP	26 Oct.	300	100	41	9	5 d	9
2,4-DP	11 Nov.	150	100	72	12	18 cd	33
2,4-DP	11 Nov.	300	100	66	6	21 cd	39
NAA	26 Oct.	200	100	57	9	52 a	96
NAA	26 Oct.	400	100	57	11	42 ab	78
NAA	11 Nov.	200	100	51	10	35 bc	65
NAA	11 Nov.	400	100	54	11	32 bc	59
LSD (0.05)						19	

Source	Pr>f
TRT	0.0001
Contrast	
Control vs. TRT	0.0023
2,4-DP vs. NAA	0.0003
Time 1 vs. Time 2	0.3109
Chemical vs. Time	0.3109
2,4-DP Concentration	0.0204
NAA Concentration	0.3273
2,4-DP Concentration x time	0.0050
NAA Concentration x time	0.5932

Figures 1a and 1b. Effect of 2,4-DP (150 & 300 mg/*l*) and NAA (200 & 400 mg/*l*) applied on 21 October 1997 on fruit retention of 'Mihowase' Satsuma (1a) and 'Marisol' Clementine (1b). [Due to the lack of differences in thinning levels between different concentrations, the data of the treatments were combined].

a



b

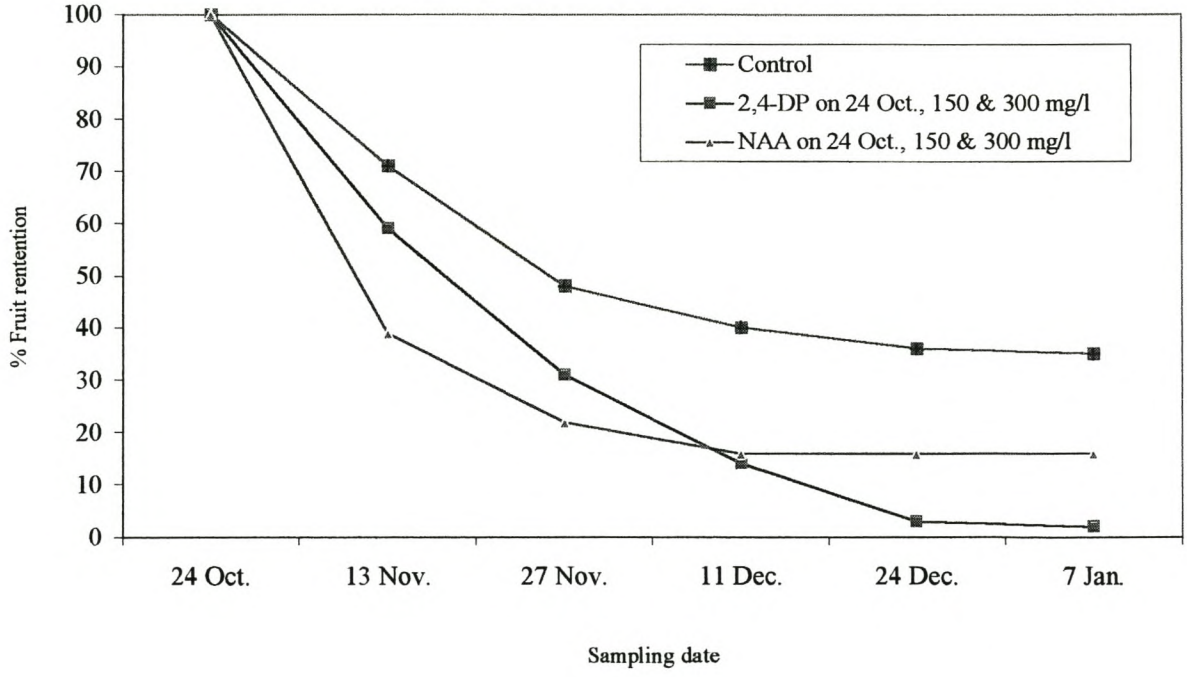
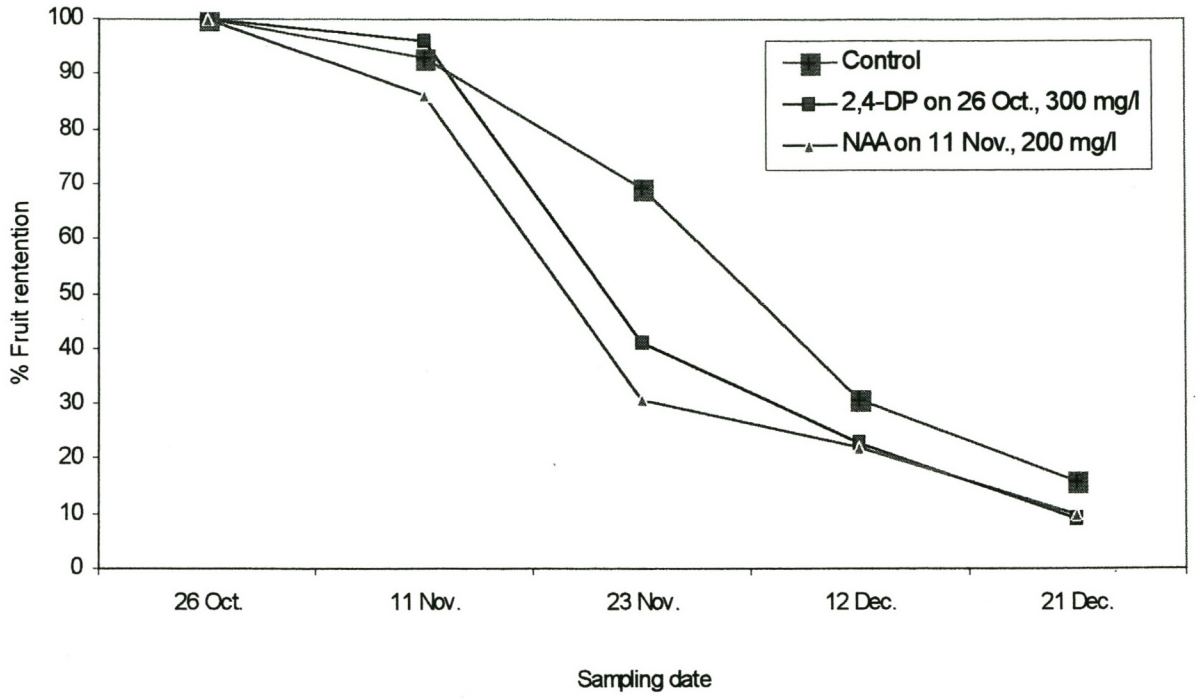


Figure 2. Effect of 2,4-DP at 300 mg/*l* and NAA 200 mg/*l* applied on 26 October and 11 November 1998 on fruit retention of 'Oroval' Clementine.



7. OVERALL DISCUSSION AND CONCLUSION

Foliar application of Progibb® (GA_3), Promalin® (GA_{4+7} + BA), Kelpak® (seaweed extract containing auxins and cytokinins) and soil applications of Temik® (aldicarb) were evaluated as a means to promote vegetative growth. GA_3 and/or aldicarb enhanced vegetative growth of 'Eureka' lemon. Therefore, GA_3 and/or aldicarb can be used as a cultural practice to promote vegetative growth, especially in cool and cold production regions, where the initial tree growth is unsatisfactory. Further studies should be conducted on other cultivars and also to conclusively show a plant growth regulator activity of aldicarb. At this stage no positive results were found with Kelpak®, possibly due to an insufficient amount of Kelpak® used, suggesting that higher concentrations should also be evaluated.

The study on carbohydrate and nitrogen reserves provided information which can be used as a basis on which to develop improved management practices in the orchard. Levels of carbohydrate and nitrogen reserves on nursery trees correspond with tree growth in the orchard following topping height, with the least increase in growth with the most severe topping treatments. Planting of large, untopped nursery trees enhanced initial tree growth in the orchard. Therefore, trees should not be topped prior to planting in the orchard.

Various branch induction techniques were conducted to enhance initial growth and tree complexity on 'Mihowase' Satsuma. The first trial, on 1-month-old trees did not respond well. While in the second trial, on 5-months-old trees, notching and girdling improved the number and total length of lateral branches, but notching was the most

effective technique. Girdling induced lateral branches, which were not satisfactory in length and were formed just within the girdled area. The trees seemingly have to be established well before they will respond. Therefore, further studies should be conducted, where notching is carried out on other cultivars of different ages and trials where several girdles along the trunk axis should also be evaluated.

Winter gibberellic acid (GA_3) and mineral oil (Bac-oil) were applied as from April until July to determine the optimum application time to reduce flowering levels and to enhance vegetative growth on 'Mihowase' Satsuma and 'Nules' Clementine. GA_3 at 50 mg/*l* in June gave the best results. Mineral oil individually does not have the desired the effect, but the combination of GA_3 and mineral oil sometimes gave good results. The optimum application time in other area and year should also be investigated, since flower initiation period may differ depending on prevailing temperature and other climatic conditions.

2,4-dichlorophenoxypropionic acid (2,4-DP) and 1-naphthaleneacetic acid (NAA) were evaluated to reduce fruit set soon after flowering and to enhance vegetative growth. 2,4-DP at 300 mg/*l* and NAA at 200 mg/*l* applied soon after flowering (fruitlet size <5 mm) was effective in fruitlet thinning. Variability in the amount of thinning was observed, but 2,4-DP was usually more consistent and effective than NAA. Measuring the response in vegetative growth would have given an idea of whether the reduction in crop load effectively advanced vegetative growth. Measuring vegetative growth responses after summer flushes should thus also be evaluated.